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THE OSTEOGENIC CAPACITY *IN VITRO* OF PERIOSTEUM AND ENDOSTEUM ISOLATED FROM THE LIMB SKELETON OF FOWL EMBRYOS AND YOUNG CHICKS

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INTRODUCTION

In the earlier work on the behaviour *in vitro* of bone and periosteum the formation of new bone *in vitro* was not observed. Bone was first cultivated by Carrel and Burrows (1910), who describe the outwandering of cells from the explant but do not record ossification in the new tissue. Similar results were obtained by Dobrowolskaja (1916), Wjereszinski (1924), working with the bone and periosteum of young rabbits, and also by Policard and Boucharlat (1925), who cultivated periosteum to which fragments of bone were attached

but failed to find osteogenesis. Dolschansky (1929), using cultures derived from the os frontalis of embryonic fowls, also states that ossification did not take place in his growths.

On the other hand, periosteal bone was found to develop *in vitro* in association with embryonic limb cartilage (Fell, 1928; Fell and Robison, 1929) and the ossification *in vitro* of mesoderm from the early embryonic jaw has also been described (Fell and Robison, 1930). According to Fischer and Parker (1929), dedifferentiated skeletal tissue which has been growing *in vitro* for many passages and has completely lost its characteristic histological structure can be made to redifferentiate into tissue which these authors regard as osteoid or chondroid in nature; this is done by diminishing proliferation by using plasma as a culture medium instead of a mixture of plasma and embryo extract. Friedheim (1930) states that bone will develop in cultures of perichondrium obtained from the limbs of rat embryos, under the influence of leprosy bacillus. Niven (1931), working on the repair *in vitro* of rudimentary limb bones from fowl and mammalian embryos, finds that new bone or osteoid tissue is formed *in vitro* at the site of fracture, and that ossification of the undamaged part of the shaft continues to advance throughout the culture period.

The primary object of the present study was to investigate the osteogenic capacity *in vitro* of the embryonic periosteum of the limb bones of the fowl at different stages of development. In a general paper on osteogenesis *in vitro* (Fell, 1931) it was briefly recorded that from the rudimentary periosteum isolated from the cartilaginous limb skeleton of 6-day embryos, bone readily developed *in vitro*. These preliminary experiments on the 6-day embryonic periosteum have been repeated and extended and work has also been done on the behaviour *in vitro* of late embryonic and early post-embryonic periosteum and endosteum. The results of these investigations are described in the present communication.

TECHNIQUE

Cultural. The hanging drop method was employed throughout this investigation. Large $1\frac{1}{4}$ inch square coverslips were used and $3 \times 1\frac{1}{2}$ inch hollow-ground slides. The culture medium consisted of fowl plasma and embryo extract.

Histological. Both cultures and controls were fixed in acetic Zenker. Heavily ossified material was hardened in alcohol after a short fixation (1 hour) in acetic Zenker and was then decalcified with formol-nitric acid. This technique gave very satisfactory results.

A few of the cultures were stained with dilute phosphotungstic acid haematoxylin and mounted whole. Others were fixed in neutral formalin and were mounted whole after treatment with either sodium alizarin sulphonate or von Kossa's silver nitrate method, to demonstrate calcification. The silver nitrate specimens were photographed immediately after mounting.

The cultures to be sectioned were not removed from the coverslip until after infiltration with paraffin wax (Fell and Robison, 1930). They were cut either parallel with or at right angles to the plane of the coverslip. Most of the sections were stained with safranin and picro-indigo-carmine, but Mallory's phosphotungstic acid haematoxylin was also used.

PART I. PERIOSTEUM

Object of experiments (Part I): To determine the osteogenic capacity in vitro *of periosteum isolated at different stages of development.*

Method

The periosteum was removed with fine needles and cataract knives from the middle third of various long bones, the ends of which were cut off and rejected. The explants were grown in a mixture of equal parts of plasma and embryo extract and were usually transferred to fresh medium every 48 hours. Throughout the culture period the central part of the culture was kept intact and at subcultivation only the periphery of the growth was cut away. The object of this procedure was to leave the middle region of the culture undisturbed and thus provide favourable conditions for organisation and differentiation.

A. *Behaviour* in vitro *of 6-day embryonic periosteum*

Material. The 6-day embryos from which the material was obtained were very well developed for their age. Tissue for explantation was taken from all the limb-bone rudiments except the tarsal and carpal elements and the digits, but the best results were obtained from the femur, tibia and humerus.

Controls. In order to determine the exact stage of development of each rudiment from which periosteal tissue was removed, one cartilage was usually taken for dissection, whilst the corresponding cartilage from the opposite limb of the same embryo was fixed and sectioned. Similarly, in order to ascertain how much of the periosteum had been explanted, the fragment of cartilage from which the tissue had been stripped was, in many cases, also fixed and sectioned. The periosteal tissue removed from each rudiment was explanted separately so that each culture had thus a double control (Plate I, figs. 1 and 2).

Serial sections of the first set of controls, i.e. the cartilage rudiments fixed entire, usually showed the following histological structure (Plate I, fig. 1 and Plate III, fig. 4). There was a middle portion occupying about one-third of the total length of the rudiment, in which the cells had begun to hypertrophy and to assume the vacuolated appearance characteristic of the chondroblasts of "ossifying" cartilage; this region of enlarged cells was continuous on each side with two regions of flattened cells, which in turn passed gradually into the small-celled areas of the future epiphysis, the articular parts of which were still composed of undifferentiated mesoderm. The first sign of osteogenesis had appeared around the middle third of the rudiment where the

perichondrium had become differentiated into two layers—an inner stratum of polyhedral cells, the early osteoblasts, and a thick outer layer of elongated cells representing the rudimentary fibrous coat of the periosteum. A very delicate sheet of osteogenic fibres was present on the surface of the cartilage immediately beneath the osteoblastic layer; in some cases osteogenic fibres also ran outwards among the osteoblasts. The two-layered structure of the perichondrium did not persist throughout the length of the shaft but became much less distinct over the area of flattened cells and disappeared on approaching the future epiphyses. In some of the controls, development was less advanced; the enlargement and vacuolation of the cells occupying the middle third of such rudiments was only very slightly marked and the layer of osteogenic fibres was hardly distinguishable; the perichondrium covering the middle region had, however, assumed its two-layered structure.

Examination of the second set of controls, i.e. the fragments of cartilage from which the periosteum had been removed for explantation (Plate I, fig. 2) showed that the region from which the periosteum had been taken usually consisted of the area of hypertrophic cells and a small part of the two zones of flattened cells. The cartilage was rarely denuded completely of osteogenic fibres and osteoblasts and in most cases all or most of the layer of osteogenic fibres and sometimes a large proportion of the osteoblastic layer were left behind. Thus the actual explant usually consisted of myoblasts and connective tissue, the rudimentary fibrous coat of the periosteum and part of the osteoblastic layer.

Observations on living cultures. Ossification centres developed in many of the cultures; in some cases cartilage appeared as well and in other specimens cartilage alone differentiated. In the most successful experiment, out of a total of thirty-six cultures, eighteen developed ossification centres and no cartilage, three formed both cartilage and bone, five gave rise to cartilage only and ten formed no skeletogenous tissue, although growth was as vigorous as in the ossifying or chondrifying specimens.

The tissue when first explanted consisted of one or more rather diffuse, irregular fragments in which the original site of attachment to the cartilage was sometimes distinguishable as a smooth semicircle.

During the first 24 hours' growth the explants usually spread out into flat plates with a very narrow, marginal fringe of amoeboid cells. Many specimens contained a compact mass of rather rounded cells which later proved to be osteogenic or chondrogenic, and from which radiated strands of myoblasts interspersed with capillary vessels and loose connective tissue; in other cultures it was impossible to distinguish different histological types.

After 48 hours *in vitro* the explants had expanded into very delicate, more or less circular sheets 1–2 mm. in diameter. In some cases the cells composing the compact areas mentioned above (Plate II, fig. 3 a) had enlarged somewhat and had assumed the appearance of osteoblasts; they contained a large spherical nucleus with one or more rounded nucleoli, showed a finely granular

cytoplasm and, as in the case of developing membrane bone (Fell and Robison, 1930), were separated from one another by delicate, slightly refractile partitions in which a fibrous structure was not apparent. These osteogenic regions were not sharply demarcated, but merged with the surrounding tissue; they varied in thickness, in some cases being as thin as the rest of the explant and in other cases forming definite nodules.

Occasionally one of the condensed areas seen after 24 hours' cultivation by the end of the second day had differentiated into very early cartilage. These cartilage nodules, which were rounded or oval in shape, differed from the ossification centres in the more regular, rounded shape of the cells and in the higher refractive index of the developing matrix which gave the nodules a characteristic glistening appearance.

Almost all the 2-day cultures showed a number of degenerate cells, the degeneration being probably due to the injury inflicted on the tissue by the original dissection.

After 4 days' cultivation most of the degenerate cells had vanished and the tissue seemed to have recovered completely. The ossification centres previously observed were considerably more advanced (Plate II, fig. 3 b). Delicate osteogenic fibres had appeared among the cells, forming rather irregular, very refractile, branching bundles. When the tissue was transplanted to fresh medium it was found that this early intercellular material had a certain amount of rigidity even in an extremely thin ossification centre. After being cut out of the plasma clot, the delicate sheet-like explant contracted greatly except in the region of the ossification centre which, though showing a few narrow creases, remained as a fairly flat plate around which the soft, contracted mesoderm was crumpled in a ring like the parchment cover of a jam pot.

Occasionally osteogenesis began as late as the 7th day *in vitro*, but most of the ossification centres were distinct by the 6th day of cultivation, although their degree of development varied considerably. Bone formation in cultures of 6-day embryonic periosteum differed from bone formation in the explants of 6-day mandibular mesoderm (Fell and Robison, 1930) in being almost invariably diffuse, i.e. the osteoblasts did not form a compact mass enclosed by a fibrous membrane, but spread out into the surrounding tissue so that osteogenic fibres radiated into the explant in all directions (Plate II, fig. 3 d). As a result almost every stage in osteogenesis could often be seen simultaneously in the same ossification centre. Thus in a 6-day culture the middle region might consist of compact osteoid tissue in which the fibrous structure of the matrix was obscured by the deposition of a refractile, interfibrillar cement (cf. the development *in vitro* of membrane bone, Fell and Robison, 1930), whilst towards the periphery only a fine network of osteogenic fibres could be distinguished among the osteoblasts.

It was interesting to note how the deposition of bone was modified by the mechanical conditions of cultivation. As stated above, narrow folds sometimes formed across the early, fibrous, semi-rigid ossification centre owing to the

contraction of the explant when removed from the plasma clot for subcultivation. The rapid expansion of the explant which always followed transplantation did not cause these folds to flatten out again and they gave rise to hard ridges which usually persisted throughout the culture period. In the larger cultures the contraction in addition sometimes formed irregular, more or less concentric folds separated from one another by comparatively flat areas of osteoid tissue (Plate II, fig. 3 a–d). These concentric folds, one of which appeared after each subcultivation, were probably due to the fact that the middle region of an ossification centre, as described above, was further developed and therefore more rigid than the fibrous outer zone, so that the latter, under the influence of the general contraction of the explant, tended to form a circular wrinkle around the former. Before the re-expansion of the explant could pull this fold flat again, advancing osteogenesis transformed it into a hard, permanent ridge.

The osteoid tissue formed in cultures of 6-day embryonic periosteum was less prone to resorption than the osteoid tissue formed from mandibular mesoderm and frequently appeared healthy after 18 days' cultivation, whilst mandibular bone which had developed under similar conditions usually began to disappear by the 12th day *in vitro*. Small ossification centres in the periosteal explants often disappeared before the 18th day, but the larger ones continued to increase in size partly by the deposition of more intercellular material and partly, as described above, by the formation of new bone at the periphery, until in some cases they reached a diameter of over 1 mm.

Calcification could sometimes be detected by the 8th or 9th day, and in good specimens was quite conspicuous by the 12th day. As in the cultures of membrane bone, calcification was seen as a deposit of brilliantly refractile granules in the matrix of the oldest, more highly developed parts of the ossification centre. Calcified areas appeared a chalky white by reflected light and almost black by transmitted light (Plate II, fig. 3 e).

In a few cultures, an ossification centre differentiated very close to the margin of the explant, some days after osteogenesis was well advanced in the other specimens; occasionally an ossification centre would form in the interior of the explant at the usual time and a peripheral one would develop several days later. It is probable that at the time of explantation osteoblasts were sometimes present at the edge of the tissue fragment and wandered into the medium in the zone of outgrowth. When the tissue was subcultivated the inner cells of the original zone of outwandering became incorporated with the main explant, whilst the outer cells again crawled into the culture medium. If osteoblasts were present in the original zone of outgrowth they would be unlikely to form bone until they had become included in the organised tissue of the culture, since cells undergoing uncontrolled growth *in vitro* seldom differentiate. This might possibly account for the delayed appearance of these peripheral ossification centres.

As described above, cartilage nodules developed in a minority of the

cultures. Such nodules were not introduced into the cultures along with the periosteal tissue at the time of explantation, but differentiated *in vitro*, all stages of chondrogenesis being seen in the living material. Sometimes cartilage and osteoid tissue occurred in the same explant, in which case the two tissues were not associated but formed separate nodules; in other specimens cartilage only was present. The cartilage generally became invested by a fibrous perichondrium, but occasionally merged directly with the surrounding tissue. Some of the nodules, usually those without a perichondrium, disappeared after a few days' growth, but the majority continued to grow and remained healthy up to the end of the culture period.

As development advanced the explants increased in thickness and the general stroma became denser and more fibrous. The original capillaries persisted as numerous branching clefts lined by endothelium, and the myoblasts were also distinguishable after 18 days' growth. Large numbers of wandering cells crawled out after each subcultivation and divided actively in the medium.

Observations on histological preparations. Histological preparations were made of most of the specimens containing ossification centres or cartilage, and of many cultures in which skeletogenous tissue did not develop. Thirty-two explants containing ossification centres only were serially sectioned and three mounted whole, ten cultures containing both cartilage and bone and eight containing cartilage only, were sectioned. Most of these cultures were controlled by sections of the corresponding long-bone rudiment from the opposite side of the same embryo and sixteen had the "double controls" described above.

The histological findings confirmed the observations made on osteogenesis in the living material.

The youngest specimen fixed and sectioned was a 2-day culture in which an ossification centre had just appeared. The osteogenic tissue consisted of an elongated mass of cells which at one end of the mass were similar to those of the surrounding stroma and at the other end were differentiated into typical osteoblasts among which were seen narrow, irregular partitions of matrix stained bright blue with picro-indigo-carmine. The rest of the explant consisted of spindle-shaped connective tissue cells, scattered myoblasts, and blood vessels which formed irregular spaces lined by endothelium and containing a few blood cells; no connective tissue fibres were seen. Mitosis was active throughout the culture.

Slightly older (3- and 4-day) specimens presented a similar appearance, but the ossification centres contained more intercellular material.

In the 6- and 7-day explants the osteoid tissue was very well developed and contained typical stellate bone cells scattered in abundant matrix; it was covered externally by a layer of osteoblasts and usually had a somewhat trabecular structure owing to the presence of large, irregular cavities lined by osteoblasts. The ossification centre was not covered by a fibrous membrane. In some cases a thin sheet of rather diffuse osteoid tissue ran out from the

main mass into the surrounding tissue. The general stroma of the explant was denser at this stage and contained intercellular fibres and sometimes compact masses of myoblasts.

Ten-day cultures (Plate II, fig. 3 f) showed little change, except that the stroma was still more dense and fibrous and the cavities lined by endothelium were more complicated.

Sections of the older (18- and 19-day) specimens showed that the ossification centres were usually still healthy (Plate III, fig. 5), and two specimens which were stained with alizarin sulphonate and mounted whole showed a fairly extensive central region of calcification surrounded by a peripheral uncalcified zone. The general stroma was loosely reticular in some regions, but elsewhere, especially in the neighbourhood of an ossification centre, it showed dense fibrous areas; the osteoid tissue was not, however, surrounded by a continuous fibrous membrane. In one specimen the ossification centre had a rather interesting histological structure. A large plate of bone had been formed, which at an earlier stage had been bent over at one end. The internal angle of this bend was occupied by a small mass of tissue having the staining reaction and histological appearance of cartilage. The bone was not deposited on the surface of the cartilaginous tissue as in the normal development of the limb skeleton, but gradually merged with it. The cartilaginous material seemed to have been formed from the stroma, with which it was internally continuous.

The isolated nodules of cartilage which, as previously described, sometimes appeared in cultures of 6-day periosteum, were found on section to be of the hyaline type and were enclosed by a fibrous perichondrium. Even in 18-day cultures the cartilage showed neither cell hypertrophy nor periosteal ossification. When a nodule of bone was present in the same culture (Plate III, fig. 6) the cartilage and bone were quite separate.

B. *Behaviour* in vitro *of* 10-*day embryonic periosteum*

Material. Material was obtained from all the long bones from one side of a 10-day embryo with the exception of the digits, and from the femur and tibia from one leg of another embryo. Several periosteal explants were made from each of the larger bones.

Controls. The corresponding bones from the opposite side of each embryo and in most cases the pieces of shaft from which the periosteum had been removed were fixed and sectioned as controls.

The embryo from which only the tibiae and femora were removed was rather better developed than the embryo from which all the long bones were taken. In both embryos the periosteum (Plate III, fig. 7) consisted of a fairly thick and tough fibrous layer overlying an osteoblastic layer which was rather irregular in thickness and somewhat diffuse along its inner surface. The periosteal bone was markedly trabecular in all the larger bone rudiments, but in the carpals and tarsals of the less advanced embryo the trabecular

structure had only just begun to appear, and the osteoblastic layer of the periosteum was consequently more compact and regular than in the larger rudiments. In the case of the more advanced embryo the cartilage was completely excavated from the middle region of the femur and tibia, but in the less developed chick excavation in this region was only partial in the larger bones and had not begun in the tarsal and carpal elements.

Sections of the fragments of shaft from which the periosteum had been dissected for explantation showed that the fibrous coat had been removed and the osteoblastic layer partially so.

Observations on living cultures. In the best experiment bone formation took place in seven cultures out of twelve, and all stages in the process of ossification could be studied in the living material.

The original explants consisted of rather tough, irregular fibrous fragments to which minute pieces of bone sometimes adhered. After 24 hours' cultivation the original fragments, which were still rather dense, had become surrounded by a zone of outwandering cells. During the next 24 hours' growth the culture began to spread out over the surface of the coverslip, whilst at the same time the fibrous tissue showed signs of disintegration, the intercellular material assuming a characteristic granular appearance indicating that resorption of the fibres was in progress. Early ossification centres were sometimes visible in 2-day cultures as rather large, compact masses of cells in which, 24 hours later, osteogenic fibres appeared.

In 4-day cultures the remains of the original fibrous tissue were still visible, but the explants were usually much more homogeneous, cellular and expanded, although a few contained a thick opaque central region in which very little histological detail could be distinguished. As a rule, the explants of 10-day periosteum did not spread out into such thin sheets as the explants of 6-day material, and in many cases it was therefore difficult to follow the stages of osteogenesis in detail. Sometimes, however, the whole course of bone formation could be observed very readily.

Most of the ossification centres were already present by the 6th day of cultivation, but in one specimen an ossification centre appeared near the periphery of the growth on the 14th day. Some of the centres were compact, i.e. they consisted of a mass of osteoid tissue covered by a layer of osteoblasts enveloped by a fibrous membrane; others were compact at one end and diffuse at the other, whilst others again were entirely diffuse. The ossification centres seemed to arise quite independently of the small spicules of bone which were sometimes accidentally included in the explant and which showed no further growth and eventually disappeared.

The ossification centres formed *in vitro* by 10-day embryonic periosteum were rather thick, and by continued deposition in some regions and resorption in others usually developed a trabecular structure. The details of osteogenesis were essentially the same as in the cultures of 6-day periosteum and need not be described.

By the 12th day *in vitro* all the ossification centres distinguishable were seen to be partially calcified. Outwandering from the margin of the explant still continued actively, with the formation of a broad zone of outgrowth, but the middle region had usually become so thick and opaque by this stage of cultivation that in several cases an ossification centre observed at an earlier period had become completely obscured.

Observations on histological preparations. Nine of the cultures containing ossification centres were fixed and sectioned, and one was stained with alizarin sulphonate to show calcification and mounted whole.

The youngest specimen fixed, which had been growing *in vitro* for 6 days (Plate III, fig. 8), contained a large oblong mass of osteoid tissue in which were several large irregular cavities filled with osteoblasts. The surface of the developing bone was covered by a compact layer of osteoblasts outside which was a continuous fibrous membrane. The rest of the culture consisted of a rather thin, dense sheet of cells and intercellular fibres, with a few small clefts lined by endothelium.

Most of the explants were fixed after 14 days' cultivation, although one was carried on until the 16th day. In these cultures the bone and osteoid tissue showed a marked trabecular structure. In the centre of the ossification centre there was usually a region which stained very deeply with picro-indigo-carmine and probably represented true bone; this was surrounded by a broad, more lightly stained zone of probably uncalcified osteoid tissue. Large, fairly dense masses of fibrous tissue were sometimes present in the inner part of the explant, which were readily distinguishable from the ossification centres.

One 14-day specimen, in which no ossification centre was present, contained a curious nodule which in life strongly resembled hyaline cartilage. In sections stained with safranin and picro-indigo-carmine this nodule was very similar to hyaline cartilage in its histological structure, and consisted of rather rounded cells embedded in an apparently amorphous matrix. On the other hand, unlike true hyaline cartilage stained by the same technique, the matrix was green in colour instead of bright pink. This was the only case in which any structure resembling cartilage was found in a culture of 10-day embryonic periosteum.

C. *Behaviour* in vitro *of late embryonic and early post-embryonic periosteum*

Material. The periosteum was obtained from the femur and tibia of a 17-day embryo, the femur of a 21-day embryo and the tibia of a day-old chick.

Controls. In each experiment the femur or tibia from one side of the body was fixed and sectioned, whilst the periosteum of the corresponding bone from the opposite side was removed and cut up into fragments for explantation. The shaft from which the periosteum had been taken was also fixed and sectioned.

Sections of the entire bones showed that the periosteum (Plate III, fig. 9) consisted of a fairly thick coat of dense fibrous tissue covering and merging

with a rather thinner layer of very flat cells. Between the flattened cells and the bone was a thin stratum of osteoblasts. The underlying bone contained numerous large, rather irregular Haversian spaces tending to radiate from the centre of the shaft and bounded by fairly thick walls of well-developed bone. The spaces were lined by a layer of osteoblasts and enclosed thin-walled blood vessels. Only the middle third of the shaft was completely filled with marrow, the future medullary cavity in the distal thirds being occupied by partially eroded cartilage.

In sections of the shafts from which the periosteum had been stripped for cultivation, it was seen that the fibrous coat and its associated layers of flattened cells had been completely removed. In some places a thin layer of osteoblasts remained attached to the bone which in other areas was covered by the remains of torn and degenerate cells; elsewhere the outer surface of the bone was completely denuded.

Observations on living cultures. The periosteum in the old embryos and newly hatched chick, which was more firmly attached to the bone than in the case of the 10-day embryos, was removed and cut into small square explants to which the ends of muscle fibres usually adhered.

A few cells emerged from the fibrous explant after about 24 hours *in vitro*, and by the 3rd day the original fragment had become surrounded by a halo of rather scattered growth. After 6 days' cultivation the scattered outgrowth had increased to form a broad sheet composed of spindle-shaped fibroblasts and a small proportion of wandering cells; the outline of the original explant appeared less sharply defined near the periphery, and the intercellular fibres were undergoing a granular disintegration.

A broad zone of actively dividing cells continued to wander into the medium after each subcultivation, whilst the cells in the interior of the culture became heaped up around the now greatly contracted original fragment, to form a central lump of organised tissue.

In most cases the culture was cut in two after 6 days *in vitro*, the two halves being placed side by side at a little distance from each other. The halves rapidly fused, and the final result of this procedure was to produce a relatively large mass of organised tissue derived from new growth, in which the original half explants were seen as small contracted islets which became smaller and more opaque as the age of the culture advanced.

Over fifty cultures were observed for periods up to 1 month, but although the majority grew profusely and appeared healthy, osteogenesis was seen in two cases only. In one culture it was noted after 9 days *in vitro* as a patch of enlarged cells embedded in refractile matrix and lying in the new growth near one half of the original explant; the specimen was fixed immediately. The other case of bone formation was not observed until the 20th day of growth, when a small fibrous mass was noticed in the outer part of the organised region. Typical osteogenic fibres began to spread from this nodule into the surrounding tissue, and by the 27th day a large kidney-shaped

ossification centre, similar to those seen in cultures of 10-day periosteum, had been formed. The ossification centre was not associated with the halves of the original explant which were conspicuous as two very opaque masses in the centre of the growth. The culture was fixed at this stage.

Observations on histological preparations. Fourteen cultures of the late embryonic (17- and 21-day) periosteum were fixed after periods of cultivation ranging from 12 to 28 days and were serially sectioned.

In the younger (12–14-day) specimens, the original explant was seen as a dense fibrous mass in the centre of the culture. It was surrounded by very cellular and much less fibrous connective tissue derived from new growth, which was sometimes compact and sometimes loosely reticular. Sections made at right angles to the plane of the coverslip showed this central region as a hump tapering on either side into the narrow zone of unorganised outgrowth. In some places, especially in the case of cultures which had been cut in half at an earlier stage, the original explant or half explant, whilst often preserving its outline intact, had begun to lose its fibrous nature and to give rise to a compact mass of cells. Mitosis was usually active throughout the culture. In some specimens numerous degenerative cells were present in the interior, but others showed little or no degeneration.

In the two oldest (28-day) cultures the fibrous character of the original fragment had completely disappeared, and only a flattened oval nodule composed of lightly staining cells was seen in the middle of the organised new growth.

Osteoid tissue occurred only in the two cultures in which it had been observed during life. The younger specimen, fixed after 12 days' cultivation, consisted of a relatively large central mass of organised new growth on either side of which lay two rounded fibrous lumps—the two halves of the original explant, which had already begun to disintegrate. The ossification centre lay in the newly formed organised tissue between the halves of the original explant and consisted of a rounded group of typical osteoblasts among which early osteoid matrix had been laid down. The surrounding tissue contained no fibres and consisted merely of a mass of healthy cells. In the older (26-day) specimen the original half explants had become reduced to small, compact areas in which no fibrous structure was seen, surrounded by a large mass of connective tissue with rather lightly straining, gelatinous intercellular material, formed by the new growth. The ossification centre which lay to one side of this fibrous tissue was much larger and better developed than that in the younger culture and the matrix stained more deeply with picro-indigo-carmine. The nodule appeared healthy and showed very few degenerate osteoblasts.

Discussion (Part I)

Two main results were obtained from the experiments recorded in Part I of this communication:

1. It was shown that periosteum in its earliest stages of development is

able to form bone even when deprived of its normal association with cartilage and of a blood and nerve supply.

2. It appeared that the osteogenic capacity *in vitro* of isolated embryonic periosteum diminishes with age.

The latter observation required explanation. The diminished osteogenic power *in vitro* might be attributed to various possible causes:

(*a*) To the total absence of osteoblasts in the original explant.

This was unlikely, since histological examination of the controls showed that the layer of osteoblasts, which is present under the fibrous membrane even in the early post-embryonic periosteum, was at least partially removed when the periosteum was dissected away for explantation.

(*b*) To a less healthy condition of the cells in cultures of older periosteum.

The majority of cultures of late embryonic and early post-embryonic periosteum, however, grew actively and appeared as healthy as the bone-forming cultures derived from 6- and 10-day embryos.

(*c*) To the osteoblasts in late embryonic life being less able to form bone under the abnormal conditions obtaining *in vitro*, than the osteoblasts from early embryos.

This possibility was supported by the fact that, on the whole, early embryonic tissue is more readily adaptable to the conditions of life *in vitro* than similar tissue from late embryos.

(*d*) To severe mechanical damage being inflicted on the osteoblastic layer when removing the periosteum for explantation, and thus preventing the multiplication and development of the osteoblasts during cultivation *in vitro*.

In late embryos and young chicks the periosteum is tough and rather firmly attached to the bone, so that such mechanical damage might easily result from stripping off the membrane. Surviving groups of osteoblasts might have little chance of recovery and differentiation, owing to the profuse overgrowth of fibroblasts from the fibrous coat.

The only way to decide which of the last two alternatives was correct was to obtain undamaged osteoblasts from the late embryonic and early post-embryonic limb skeleton and investigate their osteogenic capacity under the same experimental conditions. The experiments recorded in Part II were undertaken with this object.

PART II. ENDOSTEUM

Object of experiments (Part II): To investigate the osteogenic capacity in vitro *of undamaged osteoblasts from the limb-skeleton of late embryos and young chicks.*

Method

The endosteum of the Haversian spaces seemed ideal material for obtaining undamaged osteoblasts from late (20–21-day) embryos and young (1–2-day) chicks.

In order to obtain this tissue, the tibia was dissected from the leg of a

late embryo or newly hatched chick and the two ends of the bone were cut off and rejected, leaving only the middle third. The periosteum was then stripped off and in three of the seven experiments performed was cut up into fragments which were explanted *in vitro* as a control series of cultures. The piece of shaft from which the periosteum had been removed was split open longitudinally and the marrow was carefully scraped away. The bone thus cleaned was cut into small oblong fragments, the long axes of which were at right angles to the long axis of the tibia, as this method of cutting involved the greatest number of Haversian spaces and was found to give the best outgrowth on subsequent cultivation. The fragments were then explanted into a large drop of plasma and extract in a large hanging drop culture and incubated for 3 days, by which time a fairly broad zone of outgrowth had appeared. The cultures were cut out of the clot at this stage, transferred to fresh medium and grown for another 2 or 3 days.

After a total of either 5 or 6 days' cultivation the original bone fragment was removed from the zone of outgrowth by means of a needle and a fine cataract knife and was rejected. The ring of growth left behind was cut out of the clot and transferred to fresh medium. The cultures thus produced were maintained for various periods up to 28 days. The tissue, which was transferred to fresh medium every 48 hours, was never subdivided and only the outer part of the growth was cut away in subcultivation. Owing to the very profuse growth of these cultures and their marked tendency to liquefy the clotted medium, it was found necessary to grow them in as large a volume of culture medium as the coverslip would hold, and to make the clot rather stiff by mixing a drop of unusually concentrated embryo extract with a rather larger drop of plasma.

In the three experiments in which a control series of periosteal explants were cultivated simultaneously with the explants of bone, the periosteal cultures were treated in a precisely similar fashion to the bone cultures. There was usually more variation in the size of outgrowth in the periosteal cultures. The original explants of the most actively proliferating specimens were removed and rejected at the same time as the original explants of the bone growths, but cultures showing less active proliferation were allowed to grow for a few days longer before removal of the original tissue.

Observations on living cultures

The bone when first explanted was seen as a small, flat, rectangular mass in which the Haversian spaces were distinguishable as lighter bands running parallel with the shorter sides of the rectangle. Two days after explantation a rather sparse outgrowth composed of cells resembling fibroblasts and of a number of leucocytes and other wandering cells had appeared from the two longer sides of the explant, i.e. the two sides which cut the Haversian spaces at right angles; very little growth took place from the other (shorter) sides. The periosteal controls usually showed considerably less outgrowth at this

stage than the bone cultures. By the 3rd day the bone had become surrounded by a fairly broad zone of cells, but the outgrowth from the periosteal controls was still sparse. The cultures were then transferred to fresh medium.

Growth became much more profuse after the first subculture and by the 5th or 6th day *in vitro* the bone and many of the periosteal explants had become surrounded by quite a wide halo of cells. It was interesting to note the difference in habit of growth between the bone and periosteal cultures. The bone (Plate IV, fig. 10 a) was always surrounded by a broad sheet of irregularly shaped, compactly arranged cells forming a ring of tissue, from the periphery of which strands of elongated cells radiated into the clot. The outgrowth in the periosteal cultures, on the other hand, only rarely showed traces of a definite sheet of tissue and consisted entirely of long radiating cords of spindle-shaped cells running out from the explant in all directions.

As stated above, the original bone was removed either on the 5th or 6th day of cultivation. In many cases no trace of bone was left, but in other specimens a few minute spicules broke off from the main fragment and remained attached to the zone of outgrowth; these spicules underwent no further development during subsequent cultivation, remaining in the culture as foreign bodies. In the later experiments, owing to improvement in the manipulative technique, it was usually possible to re-explant the zone of growth after it had been cut out of the old clot, as a complete, more or less regular ring; in the earlier experiments the ring was often twisted and distorted when transferred to the fresh coverslip. The same was true of the control periosteal cultures.

As mentioned above, the Haversian spaces of a normal late embryonic tibia are lined by a layer of osteoblasts. It therefore seemed probable that the rings of tissue formed by the multiplication of cells which had crawled out from the interior of the bone would largely consist of healthy, undamaged endosteal osteoblasts which might be expected to produce bone *in vitro* if the osteoblasts of late embryos and young chicks were capable of doing so. It was interesting to find, therefore, that ***these cultures ossified in* 100 *per cent. of cases***, and bone or osteoid tissue has now been observed in over fifty specimens. None of the periosteal controls, however, showed any trace of bone formation.

For the sake of convenience the cultures derived from bone have been termed "endosteal," but this does not imply that osteoblasts only were present in the growths, which also included cells derived from the Haversian vessels.

All stages of osteogenesis could be seen in the living tissue (Plate IV, fig. 10).

When the cultures were examined about 20 hours after the removal of the bone (Plate IV, fig. 10 b) the peripheral cells were seen to be wandering outwards in the usual way, whilst the hole left after dissecting away the explant was completely or partially filled by a centripetal ingrowth of cells from the inner margin of the ring. In exceptional cases the medium liquefied in the central hole, in which case the ingrowth of cells did not take place until

the culture was changed. The periosteal controls showed the same outward and inward migrations of cells.

Forty-eight hours after dissection the hole was in most cases completely filled by cells, although its site was usually still distinguishable as a thin patch in the centre of the culture. At this stage of growth, and sometimes even earlier, the first signs of osteogenesis became visible, and when the tissue was examined under a high power a network of delicate osteogenic fibres was seen to permeate the culture. These fibres were sometimes very clearly and beautifully seen in the thin central patch. In the thick ring of tissue surrounding this area they often formed circular bands, whilst in the outer parts of the culture, where they were much more sparse, they tended to radiate towards the margin. No such fibrillary network was distinguishable in control periosteal cultures of the same age and from the same tibia.

On the 4th day following the removal of the original bone, osteoid tissue was easily recognisable in all the endosteal cultures. The matrix at this stage appeared in the middle region of the culture as an irregular mass of refractile lumps and ridges in which the original fibrous structure was almost indistinguishable. The site of the hole left by the removal of the explant seldom vanished completely, as the osteoid matrix usually remained sparse and diffuse in this area. A network of osteogenic fibres radiated outwards from the main osteoid mass into the broad sheet of organised tissue by which it was surrounded. The osteoid tissue in the middle of the culture sometimes showed thick corrugations radiating from the centre. This was a mechanical effect similar in origin to the concentric ridges described above in the case of cultures of 6-day embryonic periosteum, and was produced as follows. When the ring of new growth was removed from the original bone, the tissue, which like most forms of tissue culture had been growing under tension, contracted considerably, and if the circular sheet was broad and thin it became puckered into radiating folds when released from the clot. Before these folds could be straightened out again by the re-expansion of the tissue, they became partially ossified and persisted as rigid bars.

In the earlier experiments the endosteal cultures gave rise to osteoid tissue only—calcification was never observed. This was found to be due to an inadequate supply of culture medium and consequently in the later experiments, as described in the section on methods, the size and stiffness of the clotted drop were considerably increased with much better results. The cultures appeared healthier, grew more actively, and in almost every case formed true calcified bone (Plate IV, fig. 10 c and d). This question of the relationship of nutrition and bone development is being investigated further.

In these later experiments calcification was frequently well advanced by the 5th day after removal of the original bone fragment (Plate IV, fig. 10 c). It often progressed very rapidly, and it was not uncommon to see only a slight opacity in the matrix one day and quite a dense calcification the next. A typical periosteal control culture at this stage appeared as a flat mass of

elongated cells somewhat resembling a heap of straw and presenting a marked contrast to the regular sheet of compactly arranged cells which surrounded the new bone in the endosteal cultures (Plate IV, fig. 10 c).

As the age of the culture advanced the mass of bone in the centre enlarged, partly by the peripheral deposition of new bone and partly by increase in the quantity of intercellular material. Uncalcified osteoid tissue was often resorbed some 10 or 14 days after the removal of the original fragment, but calcified bone could persist intact for at least a month after the dissection (the longest period for which cultures were maintained).

The control periosteal cultures grew very vigorously during the first fortnight after removal of the explant, often forming a large mound of cells in the middle, and completely obliterating all traces of the hole from which the original explant was taken. A degenerate spot sometimes appeared in the centre of this lump of tissue, and some of the innermost cells were seen to be fatty or degenerate. Sometimes this rather opaque, necrotic area would spread to a comparatively large size and then at a later stage of growth would apparently be largely resorbed and eventually reduced to a small, rather hyaline nodule in which nuclei were embedded A nodule of this type was readily distinguishable from osteoid tissue.

Observations on histological preparations

The observations made on the living material were checked by histological examination of cultures fixed at various stages of growth. Most of the endosteal cultures were sectioned, but others were mounted whole after either staining with dilute phosphotungstic acid haematoxylin or treatment by von Kossa's silver nitrate method for demonstrating calcification. In all, thirteen cultures were fixed and sectioned before removal of the explant and three were stained with phosphotungstic acid haematoxylin and mounted whole, fifty-three were sectioned at various stages after removal of the original bone, and whole mounts were made of three specimens stained with Mallory's haematoxylin and of eleven treated by von Kossa's method. The periosteal controls were also histologically studied, three cultures being fixed and sectioned before removal of the explant and thirty-nine being sectioned after removal.

As previously described, bone from the middle region of a late embryonic or early post-embryonic tibia contains numerous more or less cylindrical Haversian spaces tending to radiate from the centre of the shaft; each Haversian space is lined by a layer of osteoblasts (the endosteum) and encloses delicate, endothelial vessels.

Sections of bone cultures fixed after 5 or 6 days' growth, that is, at the stage immediately before removal of the explant (Plate V, figs. 11 and 12), showed that both the osteoblasts and endothelial cells of the Haversian spaces had migrated actively into the surrounding medium. A large proportion of the actual bone cells appeared degenerate. It is possible that some of those near the cut edges of the bone or close to the Haversian spaces might have

emerged from the intercellular material and entered the culture medium, but by far the greater part of the zone of outgrowth was obviously derived from the tissue in the Haversian spaces. In one experiment in which growth had been particularly active, all three cultures fixed on the 5th day showed osteogenic fibres already formed by the new tissue around the original bone, and in two cases the fibres were quite well marked in certain areas of the culture. Osteogenesis was not observed in the living specimens before fixation, owing probably to the thickness of the zone of outgrowth in the neighbourhood of the explant. In another experiment where growth had been rather less profuse, no sign of newly formed osteogenic fibres was seen in any of the eight cultures fixed after 6 days *in vitro*.

Osteogenic fibres were present in seven out of the eight cultures fixed 14–24 hours after removal of the bone fragment (Plate V, fig. 13). They were seldom distinguishable in the living cultures at this stage, owing probably to the contracted condition of the tissue during the first 24 hours following the removal of the bone. The hole from which the bone had been dissected was filled either by a rather loose network of cells or by a plug of culture medium, and the osteogenic fibres formed a diffuse mat, stained blue with picro-indigo-carmine, in the tissue surrounding this area. Specimens cut at right angles to the coverslip were seen as a rather flat plate of tissue in which the osteogenic fibres formed either thin flat lamellae running parallel to the coverslip and sandwiched between layers of osteoblasts or fairly thick, irregular masses in which lay scattered osteoblasts.

Both the specimens fixed 3 days after removal of the bone were thicker than the 24-hour cultures, and the osteoid tissue was more abundant. In some areas the osteoid matrix was comparatively dense and deeply staining, but elsewhere it consisted merely of early osteogenic fibres.

By the 5th day after dissection (Plate V, fig. 14) the osteoid tissue in the interior of the culture had in places assumed the character of young bone, and by the 7th day (Plate IV, fig. 10 e) most of the osteoid tissue in the centre had become transformed into true bone which, as described above, was seen to be heavily calcified in life.

Eleven days after the removal of the bone fragment (Plate VI, fig. 15) quite broad and dense partitions of bone matrix had been laid down which stained a very deep blue with picro-indigo-carmine. Osteoblasts in mitosis (Plate VI, fig. 15) were still seen even in the best developed parts of the bone. In some cases the deposition of fresh bone was taking place fairly extensively at the periphery of the main mass, but usually the cultures showed signs of a diminished osteogenic activity at this stage, and the zone of newly formed osteoid tissue surrounding the central mass of bone was more restricted than in younger cultures.

One culture was fixed 21 days after removal of the original bone (Plate VI, fig. 16). The nodule of bone which had formed during this period was very well developed and healthy, but osteogenesis appeared to have stopped as

the outline of the bone was sharply marked off from the rest of the culture. The bone was surrounded by a broad, lightly staining, fibrous region which resembled ordinary connective tissue rather than osteogenic tissue.

Four cultures were fixed on the 28th day after dissection. In two of them the bone was fairly healthy, but in the other two it showed signs of degeneration.

The eleven cultures treated by von Kossa's method were fixed at periods ranging from 8 to 11 days after removal of the original bone. All the specimens showed dense blackening in the areas where calcification was suspected in the living culture (Plate VI, fig. 17 a and b).

The histology of the periosteal controls was very different from that of the endosteal cultures.

Three periosteal cultures were fixed after 6 days *in vitro*, at the stage immediately before removal of the explant. They showed a dense, deeply staining, coarsely fibrous explant, on one side of which, in one case, were tags of muscle, and from the edges of which extended the broad zone of outgrowth.

Four specimens fixed 14–24 hours after removal of the original tissue consisted of a sheet of rather loosely arranged cells among which no intercellular fibres were seen. By the 5th day the cells had become much more closely packed and the cultures had increased in thickness, forming, as previously described, a kind of lump in the centre which was very conspicuous in sections made at right angles to the plane of the coverslip. A few fine intercellular fibres, stained light blue with picro-indigo-carmine, could just be distinguished in the denser parts of the tissue.

The central lump had become relatively large 10 or 11 days after removal of the explant, and the lightly staining intercellular fibres running among the densely packed cells were more plentiful. In two cultures an area of degeneration occurred in the interior of the central mass. In one of these cases, where degeneration was less advanced, the cells in the necrotic area were highly vacuolated and in life probably contained fat. The intercellular material, which stained more deeply than in the surrounding healthy tissue, appeared as rather stout, sinuous bands running among the vacuolated cells. The other culture showed a later stage in this degenerative process. In this specimen the necrotic region was smaller and more compact; the cells were highly vacuolated and the intercellular material, which stained a dark green with picro-indigo-carmine, was less plentiful than in the healthy parts of the culture and appeared to have contracted. In two of the cultures fixed on the 12th day this type of degeneration was even more clearly shown. In the middle of these specimens was an oval nodule composed of the granular remains of cells in which somewhat shrunken nuclei were still seen, and among which ran numerous narrow, intercellular partitions of amorphous, deeply staining material representing the contracted remains of the lightly staining intercellular fibres seen in healthy cultures. This form of degeneration has

been described in some detail, as workers unused to the histology of this material might at first mistake such necrotic areas for some differentiation of a chondroid or osteoid nature.

Vacuolated, degenerate cells occurred in most cultures fixed after the 11th or 12th day following removal of the explant, although in some instances only very few degenerate cells were present and the centre of the culture was comparatively healthy (Plate VI, fig. 18).

No sign of ossification was seen even in the oldest cultures which had been maintained for 25 days after removal of the explant. Except in the necrotic areas described above, the intercellular substance remained as a lightly staining, finely fibrous material totally different from that of the bone or osteoid tissue present in endosteal cultures of the same age.

Discussion (Part II)

From the results recorded in Part II it is clear that undamaged endosteal osteoblasts from the limb skeleton of late embryos and young chicks form bone *in vitro* as readily as periosteal osteoblasts from early embryos. It might be argued that in the writer's experiments the endosteal osteoblasts formed bone *in vitro* and the periosteal osteoblasts failed to do so because the former were cultivated for some days in association with differentiated bone which in some way induced an osteogenic capacity in the surrounding cells. On the other hand, the two positive results obtained with cultures of isolated late embryonic periosteum, together with the fact that ossification took place readily in cultures of periosteum derived from 6-day embryonic limb-bone rudiments in which true bone had not yet been formed, showed clearly that contact with differentiated bone is not an essential condition for osteoblasts to manifest their osteogenic function *in vitro*.

The results recorded in Part II also fail to exclude the possibility that the capacity for bone formation, although possessed by the endosteal osteoblasts, might be partially or completely lost by the periosteal osteoblasts in the later stages of development. In this case the two positive results obtained with the late embryonic periosteum might be explained by assuming that endosteal osteoblasts had been dragged out of the Haversian spaces when the periosteum was stripped off. Histological examination of normal late embryonic and early post-embryonic limb bones shows, however, that osteogenesis is very active immediately beneath the fibrous periosteum, which makes it difficult to believe that the normal osteogenic capacity of the periosteal osteoblasts is seriously diminished at this stage.

It seems more probable that in the writer's experiments the failure of late embryonic and early post-embryonic periosteum to form bone *in vitro*, except in rare cases, was merely due to mechanical damage inflicted on the delicate osteoblastic layer when stripping off the tough fibrous covering membrane. If this interpretation of the results is correct, then the contrast between the behaviour *in vitro* of the periosteal and endosteal cultures

emphasises an important point, viz. that some essential physiological difference exists between periosteal fibroblasts and osteoblasts, since, under precisely the same environmental conditions, periosteal fibroblasts, though capable of active migration and multiplication, are incapable of bone formation, whereas undamaged osteoblasts from the same long bone are able to form bone in 100 per cent. of cases (this does not, of course, affect the possibility that under appropriate conditions fibroblasts might be profoundly altered and transformed into osteoblasts). This view is supported by Niven (1931), who found that during repair *in vitro* of fractured embryonic bones, both the fibroblastic and osteoblastic layers of the periosteum united perfectly, but that new bone was formed at the site of injury by the osteoblastic layer only.

That such perfect differentiation of bone should take place in such actively proliferating and migrating tissue as the endosteal cultures seems at first sight to contradict the view discussed by Strangeways (1924) and supported by Fischer and Parker (1929), to the effect that proliferation and differentiation are mutually antagonistic processes. It would seem, however, that this contradiction is only apparent. As mentioned above, osteogenic fibres were almost always present 12–24 hours after the removal of the original bone fragment when the tissue was in a greatly contracted state. Although this contracted ring spread out slightly during the next 24 hours' growth, the subsequent increase in area of the cultures seemed to be mainly due to the active proliferation and migration of the marginal cells to form a broad halo around the central ossifying ring which remained more or less intact. This persistence of the original ring in the middle of the culture was probably due partly to the great rapidity with which differentiation took place in the contracted tissue during the first 48 hours after removal of the original fragment and partly to the nature of the differentiation, i.e. the formation of a firm intercellular material which would tend to inhibit the spreading and proliferation of the cells and thus, by a vicious circle, would also encourage differentiation to be particularly active in this area. This view is supported by the fact that, throughout the culture period, bone formation was always much more advanced in the region of the original ring (Plate IV, fig. 10 c) and diminished as the actively proliferating and migratory zone was approached. In those cases where osteogenesis had begun before the removal of the original bone, there was always a very thick and broad zone of outgrowth present and the osteogenic fibres were best developed in the central and most dense part of this zone where proliferation and migration would be minimal.

This early restriction of growth in the middle of the culture also probably accounted for the fact that necrosis was so rare in the newly formed bone and was much more common in the centres of the actively growing, highly cellular control cultures of periosteum.

SUMMARY OF RESULTS

1. Periosteum removed from 6-day embryonic limb-bone rudiments is able to ossify *in vitro*, although by the conditions of the experiment it is deprived of its normal association with cartilage and of a blood and nerve supply.

2. Periosteum removed from 10-day embryonic limb bones is also able to form bone *in vitro*.

3. Periosteum from late embryos and young chicks may grow profusely *in vitro* but only ossifies in rare cases, probably owing to severe damage being inflicted mechanically on the osteoblastic layer when stripping away the tough, firmly attached fibrous membrane.

4. Undamaged (endosteal) osteoblasts derived from the Haversian spaces of late embryonic and early post-embryonic tibial bone readily form bone *in vitro*.

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REFERENCES

CARREL, A. and BURROWS, M. T. (1910). "Cultivation of adult tissues and organs outside the body." *J. Amer. Med. Assoc.* vol. LV, p. 1379.

DOBROWOLSKAJA, Z. (1916). "On the regeneration of bone in its relation to the cultivation of bone tissue." *Brit. J. Surgery*, vol. IV, p. 332.

DOLSCHANSKY, L. (1929). "Dauerzüchtung von Knochen- und Periostgewebe." *Zeitschr. f. Zellf. u. mikr. Anat.* Bd. VIII, S. 789.

FELL, H. B. (1928). "Experiments on the differentiation *in vitro* of cartilage and bone. Part I." *Arch. f. exp. Zellf.*, Bd. VII, S. 390.

—— (1931). "Osteogenesis *in vitro*." *Arch. f. exp. Zellf.* Bd. XI, S. 245.

FELL, H. B. and ROBISON, R. (1929). "The growth, development and phosphatase activity of embryonic avian femora and limb-buds cultivated *in vitro*." *Bioch. J.* vol. XXIII, p. 767.

—— —— (1930). "The development and phosphatase activity *in vivo* and *in vitro* of the mandibular skeletal tissue of the embryonic fowl." *Bioch. J.* vol. XXIV, p. 1905.

FISCHER, A. and PARKER, R. C. (1929). "Proliferation und Differenzierung." *Arch. f. exp. Zellf.* Bd. VIII, S. 297.

FRIEDHEIM, A. H. (1930). "Ueber Knorpel- und Knochenbildung *in vitro*." *Arch. f. exp. Zellf.* Bd. IX, S. 236.

NIVEN, J. S. F. (1931). "The repair *in vitro* of embryonic skeletal rudiments after experimental injury." *J. Path. and Bact.* vol. XXXIV, p. 307.

POLICARD, A. and BOUCHARLAT, M. (1925). "Résultats des explantations *in vitro* de périoste et de périchondre." *C.R. Soc. Biol.* XCIII, No. 26, p. 533.

—— —— (1925). "Recherches sur les explantations de périoste et de périchondre." *Arch. f. exp. Zellf.* Bd. II, S. 223.

STRANGEWAYS, T. S. P. (1924). *Tissue Culture in Relation to Growth and Differentiation.* W. Heffer and Sons, Ltd., Cambridge.

WJERESZINSKI, A. O. (1924). "Vergleichende Untersuchungen über Explantation und Transplantation von Knochen, Periost und Endost." *Virchow's Arch.* Bd. CCLI, S. 268.

EXPLANATION OF PLATES

Figs. 10 a–d, and Figs. 17 a and b are photomicrographs; all the remaining figures are camera lucida drawings.

List of abbreviations

am.c.	amoeboid cells	*Ha.s.*	Haversian spaces
b.	bone	*mi.*	mitosis
b.c.	bone cell	*n.b.*	new bone
b.m.	bone matrix	*o.f.*	osteogenic fibres
ca.a.	calcified area	*o.m.*	osteoid matrix
cart.	cartilage	*or.b.*	original bone
c.m.	culture medium	*ost.*	osteoblasts
c.t.	connective tissue	*os.t.*	osteoid tissue
fi.	fibroblasts	*r.t.*	ring-like thickenings
f.t.	fibrous tissue	*z.o.*	zone of outgrowth

Plate I

Fig. 1. Longitudinal section of a normal (control) tibia from a 6-day fowl embryo showing the general histological structure of the shaft.

Fig. 2. Longitudinal section of the middle region of the opposite tibia from the same embryo, after removal of the periosteum for explantation. The osteogenic fibres and part of the osteoblastic layer remain adherent to the cartilage.

Plate II

Fig. 3 a–e. Camera lucida sketches showing the development of an ossification centre which appeared in the culture made from the rudimentary periosteum taken from the tibial fragment shown in Fig. 2. The plate of bone which differentiated during cultivation shows three concentric, ring-like thickenings, each ring corresponding with a subculture. f. Section of the same culture fixed after 10 days' growth *in vitro*. (Plane of section at right angles to the plane of the coverslip.)

Plate III

Fig. 4. Longitudinal section through a normal (control) tibia from a 6-day fowl embryo. Note the rudimentary periosteum composed of an outer fibroblastic and inner osteoblastic coat, and the layer of osteogenic fibres. The periosteum from the opposite tibia of the same embryo was explanted *in vitro* and is represented in Fig. 5.

Fig. 5. Section of a periosteal culture made from the corresponding tibia to that shown in Fig. 4. The specimen was fixed after 18 days' growth *in vitro*. Note the bone which has developed during cultivation. (Plane of section at right angles to the plane of the coverslip.)

Fig. 6. Section of a 10-day culture of periosteum from a 6-day embryonic femur. In this specimen both cartilage and bone have developed as independent nodules. (Plane of section at right angles to the plane of the coverslip.)

Fig. 7. Longitudinal section of a normal (control) tibia from a 10-day embryo. Note the fibrous and osteoblastic layers of the periosteum overlying early bone trabeculae. The periosteum from the opposite tibia of the same embryo was explanted *in vitro* and a culture is shown in Fig. 8.

Fig. 8. Section of a 6-day periosteal culture obtained from the corresponding tibia to that shown in Fig. 7. Note the compact ossification centre which appeared during cultivation. (Plane of section at right angles to the plane of the coverslip.)

Fig. 9. Longitudinal section of a (control) tibia from a day-old chick. Note the outer coat of fibrous tissue merging with an inner layer of flattened cells beneath which lie the osteoblasts.

PLATE IV

Fig. 10 a–d. Photographs of a living culture of tibial bone from a 21-day embryo at different stages, showing the formation of bone by endosteum. The culture was obtained from the same tibia as those shown in Figs. 11, 12, 14, 15, 16 and 17.

a. Five days *in vitro*, immediately before removal of the original bone fragment.

b. Same culture, 20 hours after removal of the original bone. The hole left by the removal of the bone is still seen.

c. Same culture, 5 days after removal of the original bone. Note the mass of new bone which has developed in the middle of the tissue.

d. Same culture, 7 days after removal of original bone. At this stage the culture was fixed and sectioned.

e. Same culture, section through central mass of new bone. (Section parallel to the plane of the coverslip.)

PLATE V

Fig. 11. Section of a 5-day culture of bone from the tibia of a 21-day embryo. This explant was taken from the same tibia as the cultures shown in Figs. 10, 12, 14, 15, 16 and 17. (Plane of section at right angles to the plane of the coverslip.)

Fig. 12. Same under higher magnification. The cells composing the zone of outgrowth are mainly if not entirely derived from the Haversian spaces. Most of the actual bone cells are degenerate.

Fig. 13. Section of a similar culture 20 hours after the removal of the original fragment of bone. Note the areas of osteogenic fibres which have appeared during cultivation. (Plane of section parallel to the plane of the coverslip.)

Fig. 14. Section of a similar culture 5 days after the removal of the original fragment of bone. This specimen was derived from the same tibia as the cultures shown in Figs. 10, 11, 12, 15, 16 and 17. Two concentric rings of osteoid tissue have been formed since the removal of the original bone. (Plane of section parallel to the plane of the coverslip.)

PLATE VI

Fig. 15. Section of a similar culture 11 days after removal of the original bone. This specimen was derived from the same tibia as the cultures shown in Figs. 10, 11, 12, 14, 16 and 17. Note the well developed bone which has differentiated during cultivation. A mitotic figure is seen in one of the bone cells. (Plane of section parallel to the plane of the coverslip.)

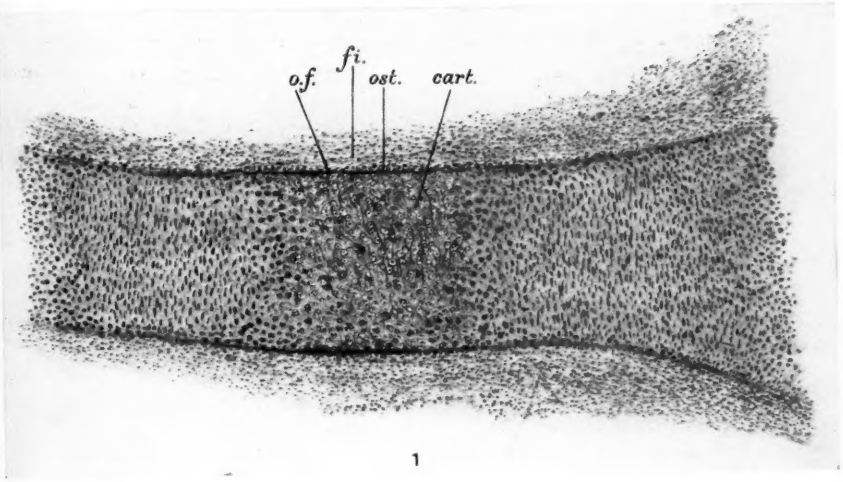
Fig. 16. Section of a similar culture 21 days after removal of the original bone. The culture was derived from the same tibia as the specimens shown in Figs. 10, 11, 12, 14, 15 and 17. A well developed nodule of bone has formed during cultivation. Osteogenesis has ceased, as indicated by the sharp outline of the bone. (Plane of section parallel to the plane of the coverslip.)

Fig. 17. Photographs of two cultures treated with von Kossa's silver nitrate method. Note the dense blackening in the newly formed bone, indicating calcification. These specimens were derived from the same tibia as the cultures shown in Figs. 10, 11, 12, 14, 15 and 16.

a. Fixed 8 days after removal of the original bone (whole mount).

b. Fixed 10 days after removal of the original bone (whole mount).

Fig. 18. Section of a culture of periosteum from the tibia of a day-old chick. This specimen was cultivated for 6 days after which the original explant was removed and the zone of growth was cultivated for a further period of 14 days. No sign of osteogenesis is seen and only fibrous tissue has been formed. (Plane of section parallel to the plane of the coverslip.)



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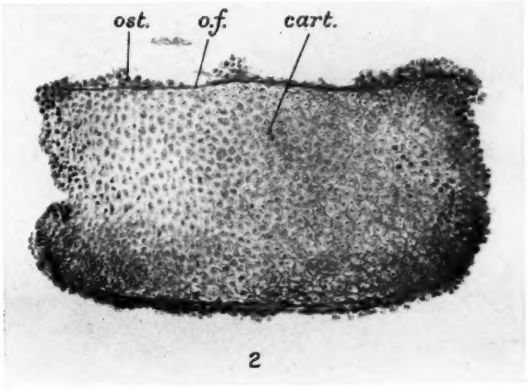

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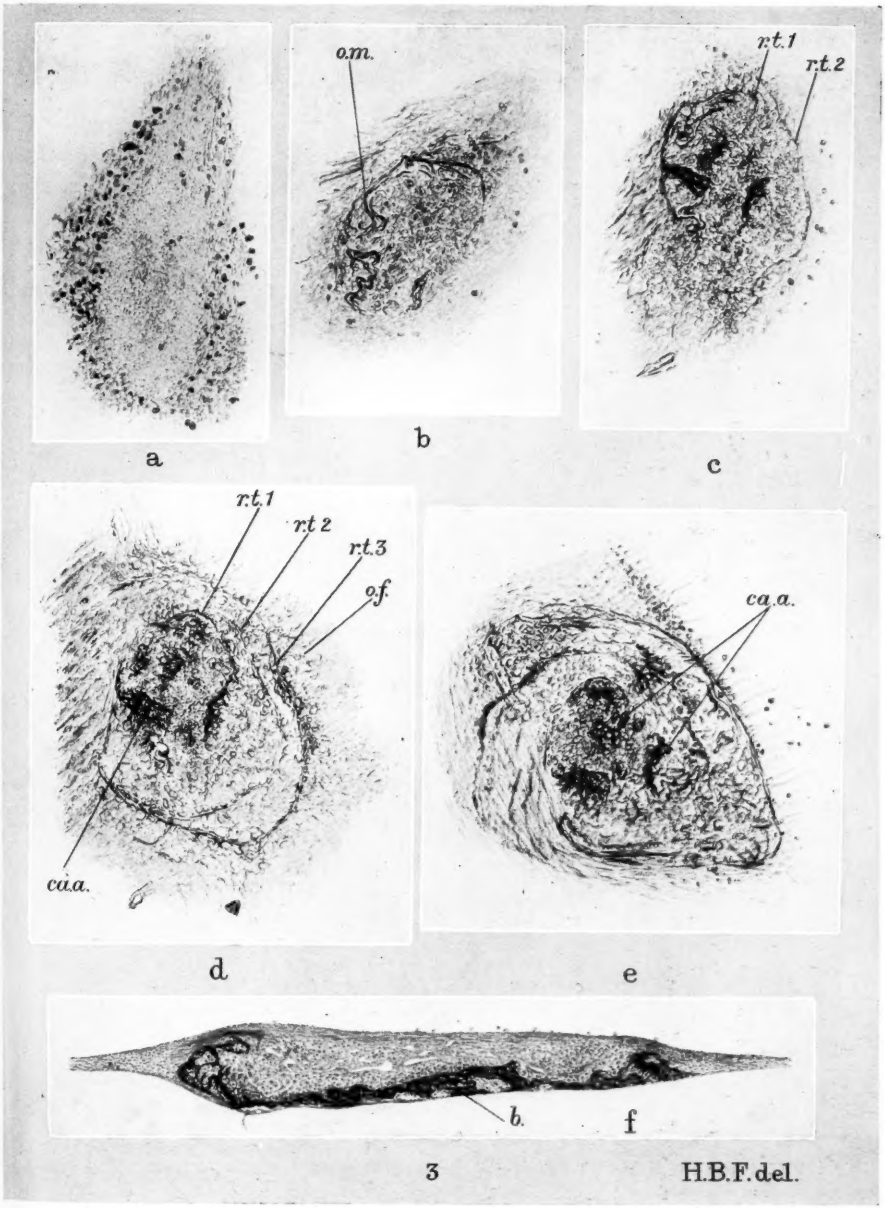

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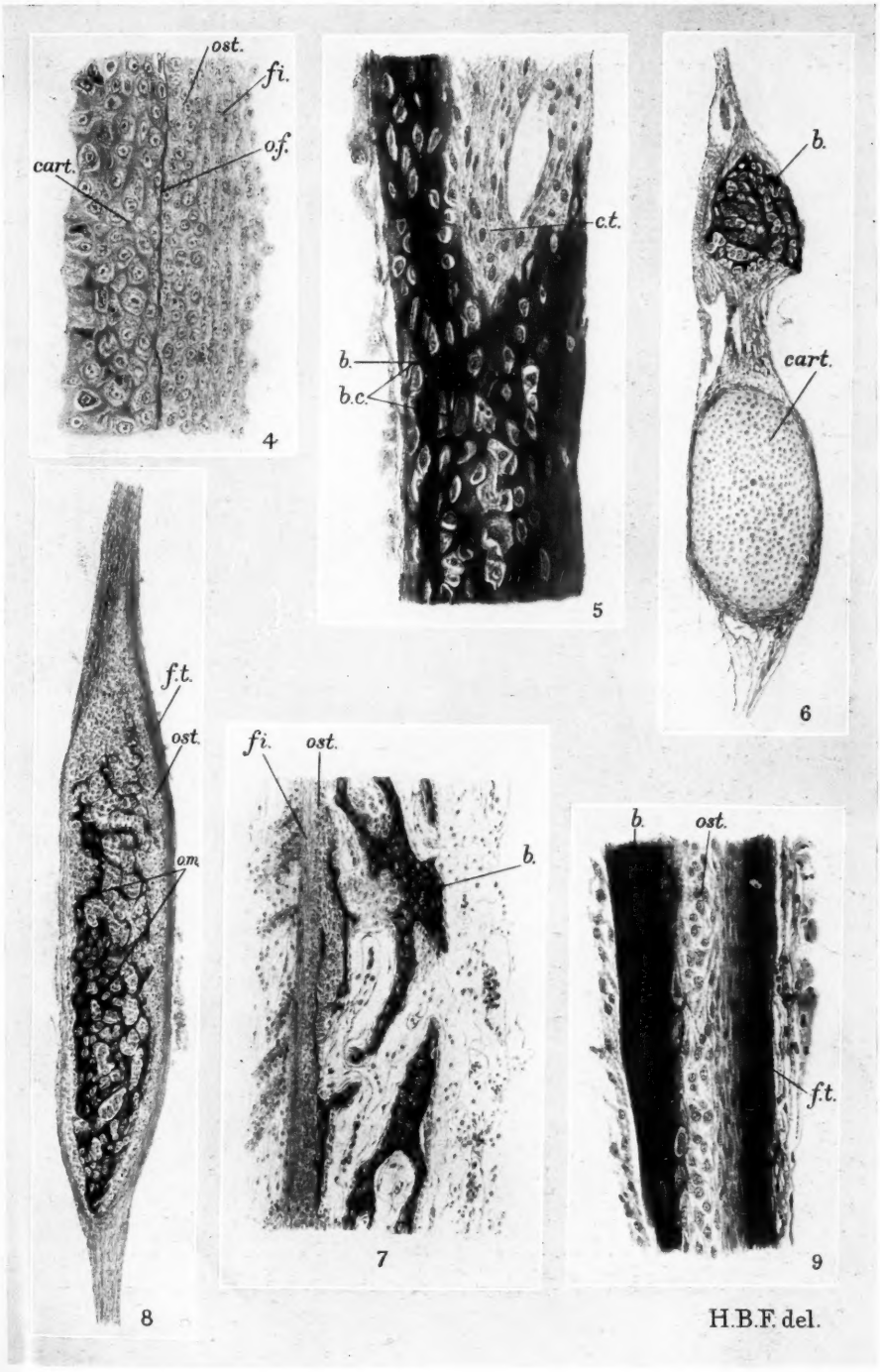
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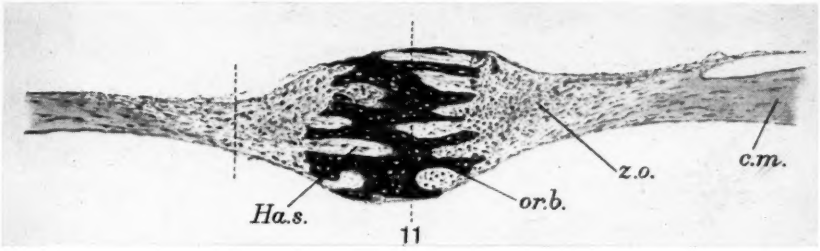

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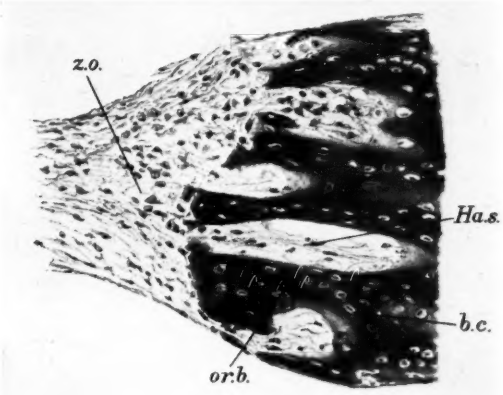

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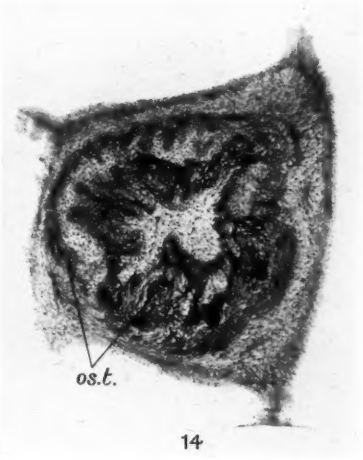

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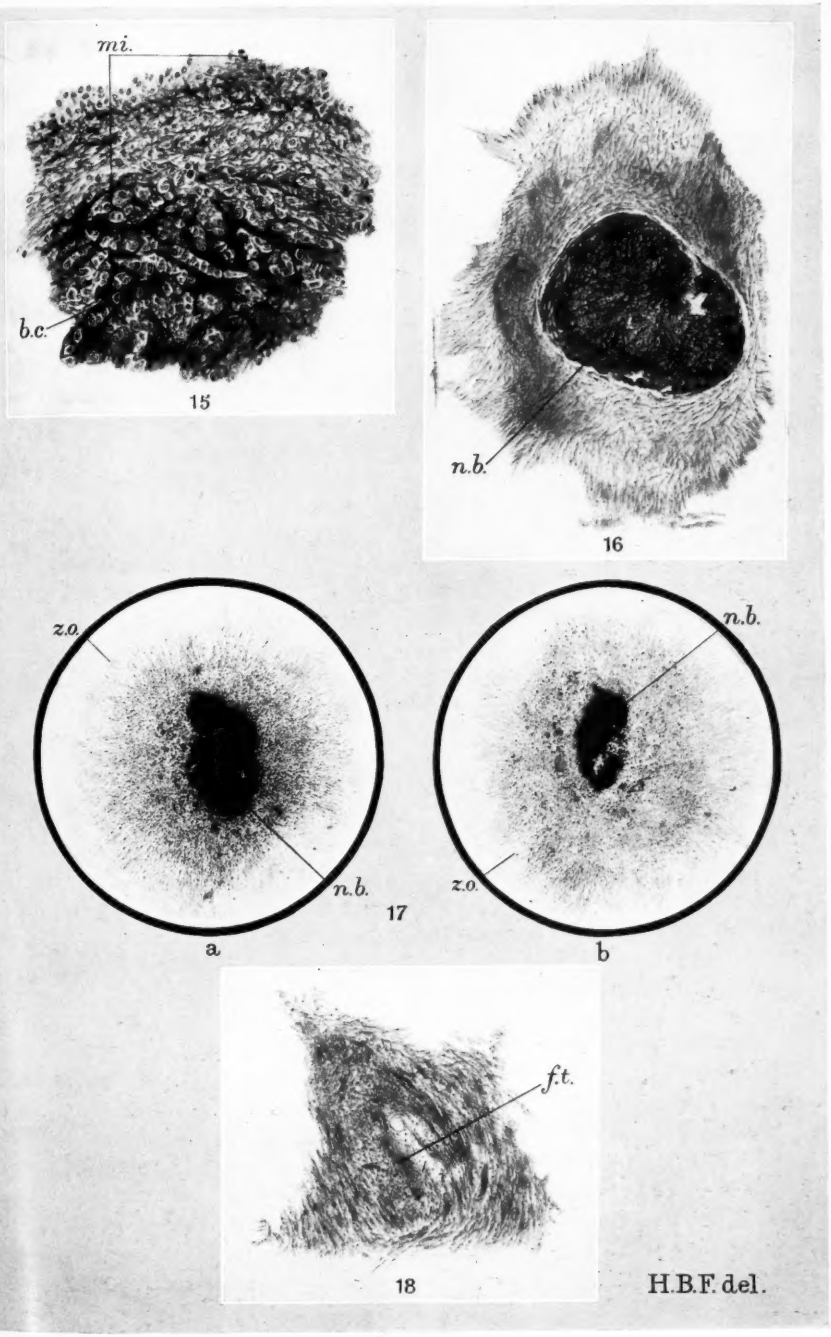

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THE UTERUS OF *CAVIA* AFTER INTRAVITAM STAINING WITH TRYPAN BLUE

PRELIMINARY NOTE

By THOMAS NICOL, M.B., Ch.B., F.R.C.S.E.
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In the course of observations on the behaviour of the tissues of *Cavia* to intravitam staining with trypan blue, my attention was arrested by the very vivid staining of the mucous membrane of the uterus, and the large size of the blue-stained granules which were seen in certain large mononuclear cells therein. Further observations seemed to show that this intense staining was localised, and that it varied in intensity and in extent in different specimens. The matter was pursued in the hope that intravitam staining might supply some new data regarding the activities of the endometrium in the oestrous cycle, and in the hope further that some light would be thrown on the character of the cells containing the blue dye.

A large amount of material has been accumulated during the past two years and many observations made both in non-pregnant and pregnant animals. The present paper is a brief preliminary account of some of the main observations in the non-pregnant animal.

The oestrous cycle in *Cavia* is normally one of about sixteen days (Stockard 1917, p. 243), but may be lengthened a little by cold. It is generally recognised that very soon after parturition guinea-pigs are in a condition of heat (Stockard, 1917, p. 227), inasmuch as they accept the male, and, if mated, pregnancy may take place; if they are prevented from mating and the young removed, the normal oestrous cycle is established and heat will recur in or about the normal time of sixteen days after parturition, as can be verified by the vaginal smear method and microscopic examination of the endometrium.

A series of unimpregnated animals after parturition were injected with trypan blue and killed at different intervals of time after parturition. Each animal was treated with similar amounts of the dye according to a weight basis, and received the same number of injections before being killed, the technique of preparation of the sections being the same in all cases.

At three and a half days after parturition practically no dye is seen in the endometrium with a low power lens, but a few granules are observed with the high power scattered irregularly throughout the mucous membrane.

At six days after parturition there is the same absence of visible dye under a low power, and again a few granules are seen with a high power scattered irregularly in the endometrium.

At nine days after parturition a marked change is to be observed. A considerable amount of dye is now present. The staining is for the most part subepithelial, and a striking feature is that the dye is in increased amount around the anti-mesometric half of the lumen, and especially at the anti-mesometric side, the granules being larger here and extending out into the stratum compactum as far as the edge of the stratum spongiosum (fig. 1). There is very little dye around the mesometric half of the lumen and practically none at the mesometric side.

At the twelfth day after parturition these appearances are distinctly intensified. The amount of dye is greater and the anti-mesometric incidence is even more definitely established (fig. 2), and as before only a small amount of dye being present around the mesometric half of the lumen (fig. 3). The dye is carried in large round cells, the structure of which is completely obscured by the blue granules, but the cell outline is well defined and perfectly sharp (fig. 4). These dye-carrying cells are large mononuclear cells with an oval or sausage-shaped nucleus (fig. 5). The blue granules are chiefly large and coarse, scattered irregularly throughout the protoplasm, and in the intensely stained cells they tend to fuse and may obscure the nucleus.

At sixteen days after parturition the dye is slightly increased in amount and its location at the anti-mesometric side is still more emphasised. The animal, as to be expected, is in heat, and the epithelium is high and columnar, shows invasion by leucocytes, and the endometrium is very vascular. Most of the cells carrying the dye are now irregular in outline, and it would appear as if the dye was being shed (fig. 6). A few granules of the stain are now seen in the epithelial lining. The cells on the edge of this coloured zone are not so deeply stained. That these intensely stained cells with irregular outline are of the same nature as the large regular mononuclear cells was proved by bleaching the sections with permanganate of potash and sulphurous acid, and re-staining with haematoxylin and eosin. They lie in the stroma without apparently any relation to the glands or blood vessels.

At twenty-one days after parturition, the staining is less intense and the cells containing the dye are scattered irregularly throughout the endometrium. The cells containing the dye are not collected close to the lumen, as in heat.

After this the staining becomes still less pronounced, and the phases described in the earlier stages are restored.

These observations seem—whatever be the interpretation of intravitam staining—to indicate a localisation of oestrous cycle changes of greater intensity around the anti-mesometric half of the lumen and especially at the anti-mesometric side, i.e. over the implantation zone.

These experiments were repeated on virgin animals, the series being established by determining the day of heat by the vaginal smear method (Stockard, 1917), and the vascular and epithelial appearances in the uterus. Practically identical results were obtained.

This endometrial reaction to trypan blue was not found in sexually im-

Fig. 1. Section of anti-mesometric side of uterine lumen to show the distribution of the dye at nine days after parturition. Unimpregnated guinea-pig aged six months. Photomicrograph. Fixation formalin and corrosive sublimate. Stained carbol-fuchsin. × *circa* 112.

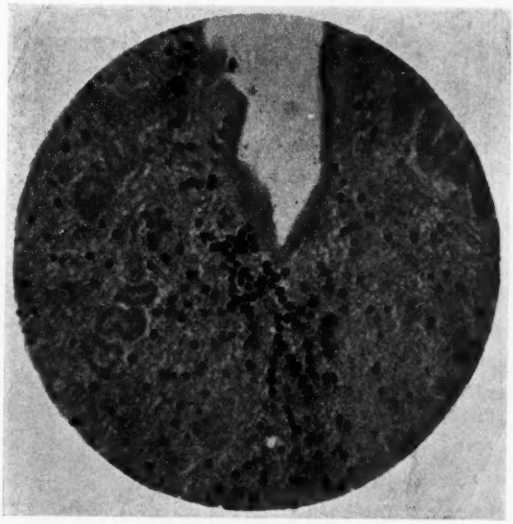

Fig. 2. Section of anti-mesometric side of uterine lumen to show the distribution of the dye at twelve days after parturition. Unimpregnated guinea-pig aged six months. Photomicrograph. Fixation formalin and corrosive sublimate. Stained carbol-fuchsin. × *circa* 112.

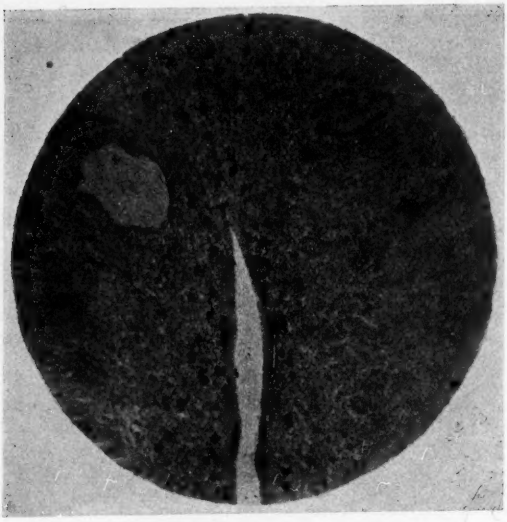

Fig. 3. Mesometric side of uterine lumen of the same section as fig. 2. Photomicrograph. Compare the distribution and the amount of the dye. × *circa* 112.

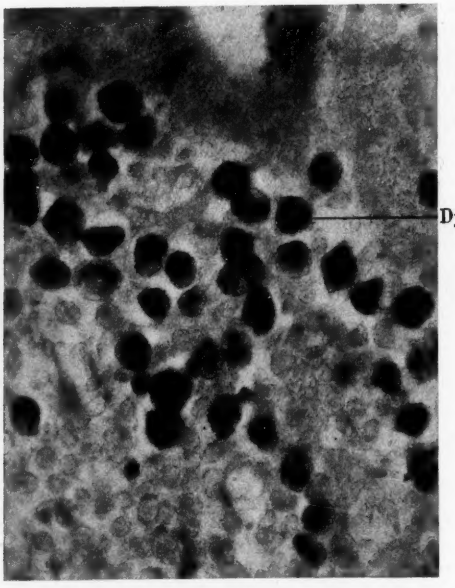

Fig. 4. Anti-mesometric side of uterine lumen of same section as fig. 2. Photomicrograph. The dye-carrying cells are figured black. × *circa* 506.

mature animals. Out of a series of six virgin pigs of known age of eight weeks, the endometrium was practically devoid of dye in five. In these five pigs both ovaries when cut serially showed no evidence of corpora lutea. In the sixth of this series the special reaction was present, but the ovaries of the animal when cut serially showed a well-marked corpus luteum in one ovary, and none in the other. The fact that this animal had ovulated, and the appearance of its uterus, seemed to justify the conclusion that this animal was sexually mature.

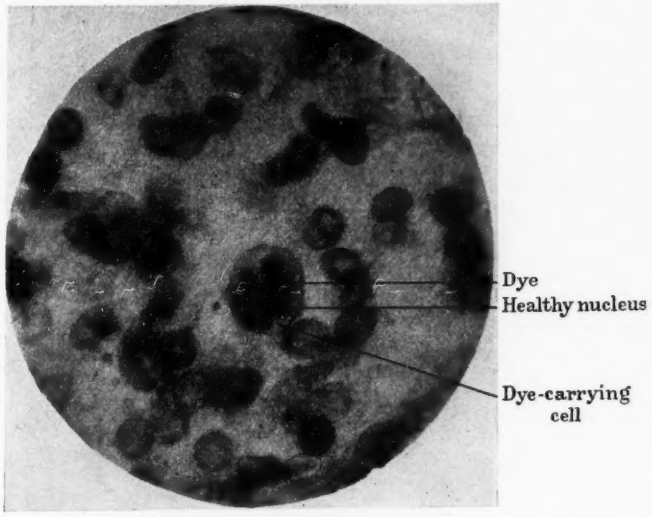

Fig. 5. Photomicrograph of the endometrium of the anti-mesometric side of the uterine lumen at the edge of the stratum compactum to show the form of the cell containing the dye. The nucleus of the dye-carrying cell is healthy and free of dye. Unimpregnated guinea-pig aged six months at sixteen days after parturition. Fixation formalin and corrosive sublimate. Stained carbol-fuchsin. × *circa* 844.

These results on immature animals seemed to confirm the conclusion that the deposition of the dye is related to a phase in the activity of the endometrium during the sexual cycle, and strengthen the deduction that was made regarding its anti-mesometric incidence. It may here be noted in this connection that the vaginal mucosa shows a lesser deposit of dye than the uterus, and the distribution is uniform around all parts of the lumen. Further, there is at no time in the cycle any dye reaction in the mucosa of the Fallopian tube.

The fact that the specificity of the dye reaction around the anti-mesometric half of the uterine lumen extends equally along the whole length of the uterine horns, as proved by serial drawings, indicates that this method does not demonstrate any special sites for implantation of the blastocyst.

It has been shown then that the blue dye affects large mononuclear cells

from some of which the dyed material appears to be shed at heat, and the question is the nature of these stained cells:

1. Are they enlarged endometrial cells laden with some chemical substance destined after implantation of the blastocyst for the supply of nourishment to the embryo?

2. In view of the enormous amount of destruction which takes place immediately after implantation, are these cells related rather to destructive than

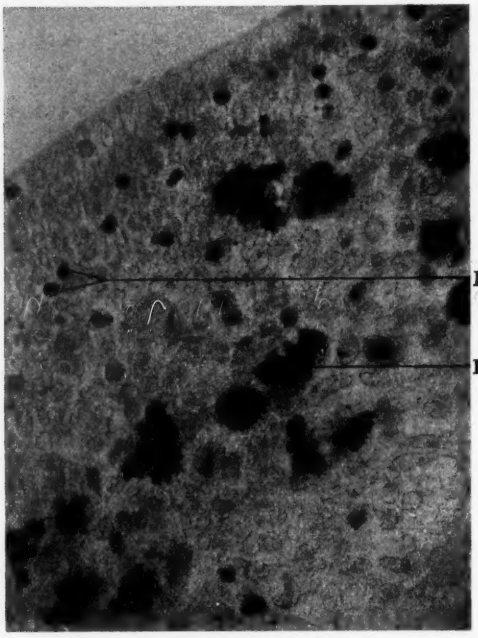

Fig. 6. Section of anti-mesometric side of uterine lumen of an unimpregnated guinea-pig aged six months at sixteen days after parturition. This guinea-pig was in heat. Photomicrograph. The dye-carrying cells figured black are irregular and appear to be shedding the dye. Fixation formalin and corrosive sublimate. Stained carbol-fuchsin. × *circa* 506.

to constructive processes, e.g. enzyme formation responsible for tissue destruction or digestion? In passing it may be noted that the destruction space is filled in the earlier stages with plasmodial masses of maternal origin (Maclaren, 1926), and later the space shows an apparent coagulum.

3. Are they cells which wander, like the large mononuclear cells found in great numbers in the resolution phases of peritoneal inflammation, and which experimentally have been shown to take up vital dye, and which generally are regarded as macrophages (Cappell, 1930)?

The work so far as it has proceeded does not warrant a definite conclusion

on these points, nor does it yield an explanation for the anti-mesometric incidence of the dye-bearing cells, but it is hoped that experimental operative work now in progress, and the finding of what the reaction is in the very early stages of implantation may throw some light on the interpretation of these results.

Although not directly related to the observations described in this preliminary note, an observation may be recorded here, because it may perhaps have some bearing on the nature of the reaction of the living cells to trypan blue. In a series of sections of the uterus of a pregnant guinea-pig, the embryo being a maximum age of eighteen days and a maximum length of 2·5 mm., the presumably endoderm cells of the yolk sac showed an abundant deposit of the blue dye. The staining is most intense in the dorsal third of the so-called endoderm where the cells are columnar, the dye granules being very large and coarse and confined chiefly to the deeper parts of the cells. As the cells become progressively flatter the blue granules are not so numerous but are still large and coarse. The intense staining begins near the attachment of the so-called yolk-sac endoderm to the edge of the developing placenta and ends a short distance from the embryo. The embryonic endoderm is entirely free of dye. The dye could not have come from the maternal circulation as the foetal and maternal circulations were not yet in contact, the allantois in this embryo not having reached the placental site. In this case the blue staining material can only have been absorbed from the destruction space by the so-called endoderm cells of the yolk sac.

The ectoplacental endoderm is apparently free of dye, though an occasional small granule is found in the cells near the attachment of the presumably yolk-sac endoderm to the edge of the developing placenta. No granular staining was found in the developing placenta, but occasionally small apparently foetal plasmodial masses near the surface of the placental labyrinth showed a very pale diffuse blue coloration. In the foetal syncytial masses which invade the decidua and form an irregular boundary between the foetal and maternal tissues, commonly referred to as the central zone or "Umlagerungszone," no granular or diffuse staining was found.

Wislocki (1921), working on older embryos of a minimum length of 15 mm. in which the foetal and maternal circulations are in contact, also found the so-called endoderm of the yolk sac stained, but no dye in the ectoplacental endoderm. He states that the dye was stored to a slight extent by the ectoderm covering the chorionic villi of the placental labyrinth, but was not taken up by the interlobular syncytium, or by that part of the ectoderm composed of giant cells and syncytium which occupies the central zone (Umlagerungszone). He also found the dye in the form of fine granules in the layer of giant cells of Duval situated beneath the ectoplacental endoderm. He states with reference to no dye being found in the central zone "this is surprising, since in the injected mouse and rat these cells are filled with blue pigment throughout gestation. Possibly the fetal syncytium stains vitally in the guinea-pig in

younger stages, when the ectoplacental cone is sending out syncytial roots and is burrowing its way into the decidua. However, in the youngest fetus (15 mm.) observed in these experiments no dye was found, nor was any found in the syncytium which, in the guinea-pig, divides the placental labyrinth into numerous lobules, the so-called *interlobular* syncytium."

Working with much earlier stages, we find that in an embryo of a maximum length of 2·5 mm. and a maximum age of eighteen days, where the allantois is not yet in contact with the developing placenta, there is no dye in the foetal syncytium of the central zone, and in still earlier embryos of a maximum age of twelve days both the developing placenta and the so-called yolk-sac endoderm are entirely free of dye. The dye only begins to appear in the yolk-sac endoderm in an embryo of a maximum age of fifteen days.

REFERENCES

Stockard, C. R. and Papanicolaou, G. N. (1917). "The existence of a typical oestrous cycle in the guinea-pig—with a study of its histological and physiological changes." *Amer. J. Anat.* vol. xxii.

Maclaren, N. (1926). "Development of *Cavia*: implantation." *Trans. Roy. Soc. Edinburgh*, vol. lv, pt 1, No. 5.

Cappell, D. F. (1930). "Intravitam and supravital staining: IV. The cellular reactions following mild irritation of the peritoneum in normal and vitally stained animals, with special reference to the origin and nature of the mononuclear cells." *J. Path. and Bact.* vol. xxxiii.

Wislocki, G. B. (1921). "Further experimental studies on fetal absorption. III. The behavior of the fetal membranes and placenta of the guinea-pig toward trypan blue injected into the maternal blood-stream." *Contributions to Embryology*, vol. xiii, No. 62; *Carnegie Inst. Wash. Pub.* No. 276.

(Wislocki's paper is a further contribution to the earlier work of Goldmann on the rat and mouse: Goldmann, E. E., "Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der 'vitalen Färbung'." *Beitr. z. klin. Chir.* vol. lxiv, p. 192, 1909.)

A MONSTROUS TWIN EMBRYO IN A LIZARD, *TILIQUA SCINCOIDES*

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I. INTRODUCTION

While dissecting some Australian Blue-tongued Lizards (*Tiliqua scincoides*), a pregnant female was encountered bearing eight ova in an early stage of development. Each oviduct contained four eggs of normal size, i.e. approximately 2·2 cm. in diameter, and each ovary contained four prominent haematoma-like areas each about 6 mm. in diameter, which proved microscopically to be developing corpora lutea.

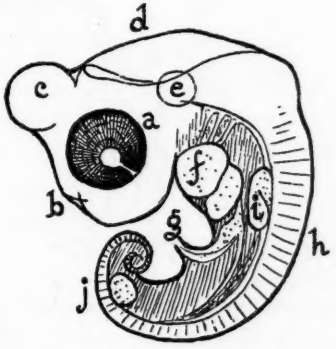

Fig. 1. A normal embryo seen through a hand lens. × 8.

Each of the eight ova still consisted largely of yolk, but presented at one pole a transparent amniotic cavity 8 mm. in diameter. On opening the amniotic sacs, seven of the contained embryos were normal, but the eighth ovum contained a twin monster.

The appearance of the normal embryos is indicated in fig. 1. The uncoiled cranio-caudal length, measured from the roof of the hind-brain to the convexity of the back just proximal to the lower limb buds, was 5·5 mm. The maximum antero-posterior diameter, measured from the fore-brain to the dorsum just cranial to the level of the upper limb buds, was 4·5 mm. The maximum transverse width, from cornea to cornea, was 2 mm. The following features were visible through a hand lens:

(*a*) Darkly pigmented eyes, each 1 mm. in diameter, with a pupil 0·3 mm. in diameter, and a distinct choroidal fissure extending dorso-caudally from the pupil.

(*b*) Fore- and mid-brain, faintly demarcated from each other by a shallow depression.

(*c*) Prominent spherical corpora bigemina 1 mm. in diameter.

(*d*) A thin-roofed hind-brain with transparent walls enabling one to see clearly the contour of the floor of the ventricle.

(*e*) Faintly visible otic vesicles on either side of the hind-brain, each 0·5 mm. in diameter.

(*f*) A prominent heart, with distinct dark auricle and pale ventricle, and three branchial arch vessels visible, extending from the heart region dorsally to the dorsal aorta.

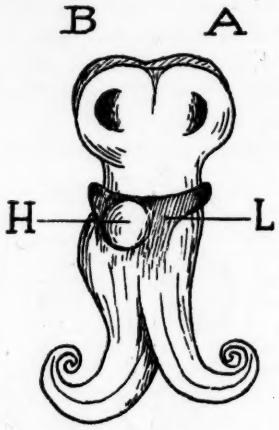

Fig. 2. The malformed twin embryo seen through a hand lens. × 8.

(*g*) Vitelline vessels emergent in the body stalk and communicating with a dark vascular hepatic anlage just caudal to the heart.

(*h*) Distinct somites extending from the hind-brain region to the coiled tail.

(*i*) Upper limb buds of two segments arising opposite the ventricle of the heart and projecting laterally and caudally from the body.

(*j*) Lower limb buds, doubtfully segmented, arising at the root of the coiled tail, and projecting laterally and ventrally from the body.

The seven normal embryos were embedded in paraffin and serially sectioned, some frontally, some sagittally and some transversely. A study of these series of sections confirmed the normal structure of the seven embryos, and, though these specimens were of value in identifying parts of the abnormal embryo, no further description of them is here necessary.

The monstrous twin, viewed with a hand lens, is depicted in fig. 2. It consisted apparently of two embryos, A and B, imperfectly developed and partially

fused back to back. Its cranio-caudal length was 6 mm. The cephalic region presented four hemispherical projections each bearing a pigmented area evidently representing an imperfectly developed eye. No distinct pupils or choroidal fissures could be discerned, and the pigmentation of each eye was less well defined than in the normal embryos and did not form a complete circle. No indications of the various regions of the brain, as seen in the normal embryos, could be discovered. A clear vesicular projection, *H*, was present on one aspect of the monster in the cardiac region. Situated one on each side of this were two smaller evidently blood-containing projections joined by a narrow bridge cranial to the vesicle, *L*. Caudally the monster consisted of two diverging coiled tails. It was observed with some surprise that neither limb buds nor somites could be discovered macroscopically. No well-defined body stalk could be seen, but some delicate membranous strands of tissue clung around the caudal parts of the monster.

Serial paraffin sections 10μ thick and numbering 600 were prepared and stained by haemalum and eosin. The following descriptions are based on a study of these sections and of partial reconstructions made from them.

II. DESCRIPTION OF THE DOUBLE MONSTER

The accompanying photographs and diagrams explain more lucidly than words the remarkable structure of the malformed twin embryo, a general summary of which may, however, be made under the following heads.

(1) *The entodermal epithelia*

In the sections of the upper half of the monster (figs. 3–5) it is at once apparent that this is clothed externally not by ectoderm but by alimentary entoderm. This is evident, not only from the character of the epithelium itself, but by its relationship to the notochords and to the hepatic rudiments, and by the absence of any entodermal derivatives centrally situated within the double embryo.

In the cranial region the otherwise complete entodermal covering is interrupted by the presence of open gill clefts, three pairs in one twin and two pairs in the other (figs. 3 and 4, *G*). The four highest or first clefts are widely dilated by the partial protrusion through them of the eyes, a remarkable relationship to be discussed later. Another deficiency in the cephalic entoderm of each twin (not seen in the figures) is a slit-like communication with Rathke's pouch. This aperture is of course due to the absence of the primitive bucco-pharyngeal membrane which otherwise would still separate the entodermal covering from the stomodeal region. It may be noted here that the bucco-pharyngeal membrane had completely disappeared in all the normal embryos. Clearly in the cranial part of the monster the investing entoderm must have been subjected to great mechanical distension by the growth of the centrally situated brain, eyes and other structures, and the resulting attenuation of the layer of alimentary epithelium is evident in figs. 3–5.

Sections in the cardio-hepatic region present a less complete entodermal investment. While the whole of one aspect of the twin body (the reverse of fig. 2) is completely clothed by entoderm, mesodermal structures, namely the serosal covering of the liver and heart of twin *A*, appear externally on the aspect presented in fig. 2. From figs. 5 and 6 the visible projections in fig. 2

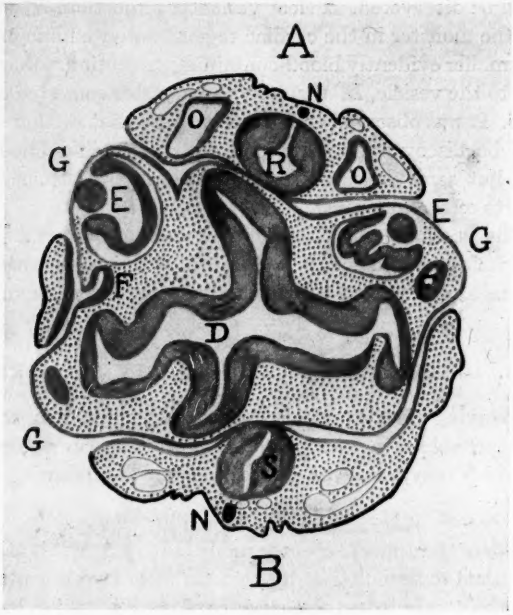

Fig. 3.

Figs. 3 to 9. Diagrams of cross-sections of the double monster, indicating the identity of the various parts. Ectodermal structures are blue, entodermal structures black, and mesodermal structures stippled. A and B denote the two members so specified in fig. 2, and the following symbols are used throughout: *C*, coelom; *D*, fused diencephalic rudiments (compare figs. 10 and 11); *E*, eye; *F*, olfactory pit; *G*, gill cleft; *H*, heart; *J*, lung bud; *K*, kidney; *L*, liver; *N*, notochord; *O*, otocyst; *P*, pancreas; *Q*, central amniotic space; *R*, hind-brain; *S*, spinal cord; *T*, upper limb bud; *W*, tubular hind-gut; *X*, torn margin of splanchnopleuric membrane. In cranio-caudal order, the following sections are illustrated: fig. 3, section 140; fig. 4, section 180; fig. 5, section 220; fig. 6, section 240; fig. 7, section 300; fig. 8, section 360; fig. 9, section 400. Magnification of all sections, 25 diameters.

may clearly be identified as the liver and heart of twin *A* protruding at the upper angle of deficiency of the entodermal covering. At the margins of this deficiency the entoderm does not cease, but is reflected off the body of the monster along with a thin mesothelial layer continuous with the coelomic lining. This reflected splanchno-pleuric lamina forms a delicate membrane which

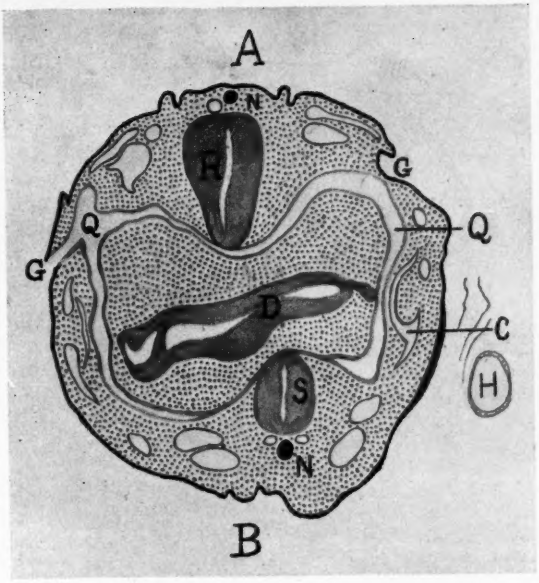

Fig. 4.

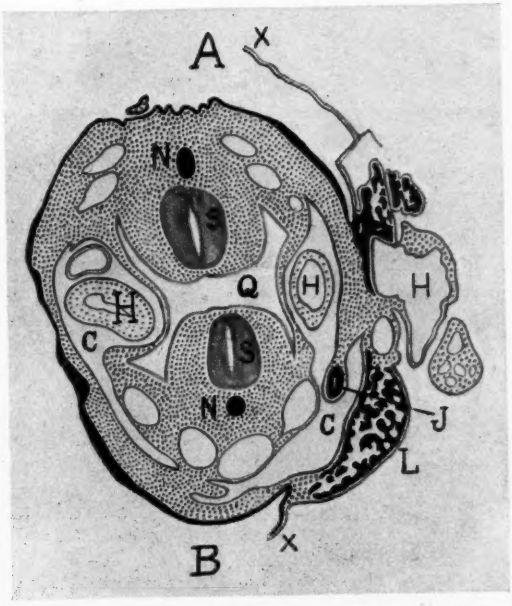

Fig 5.

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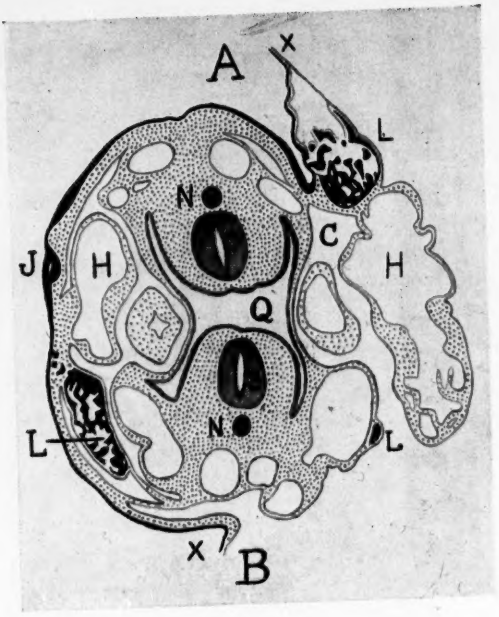

Fig. 6.

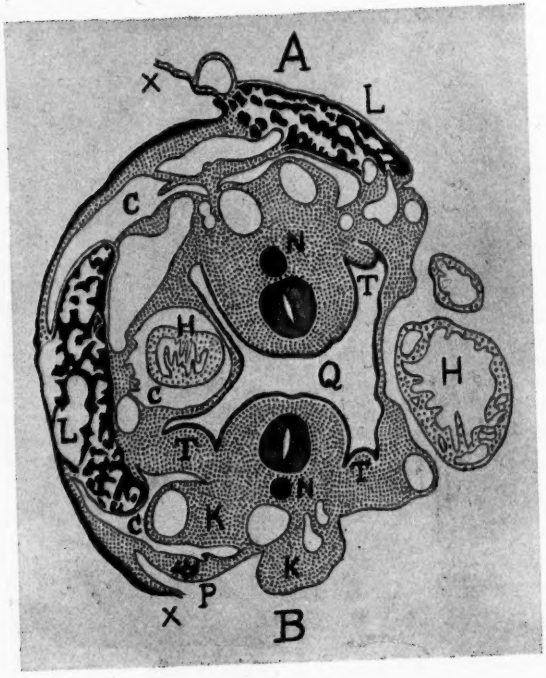

Fig. 7.

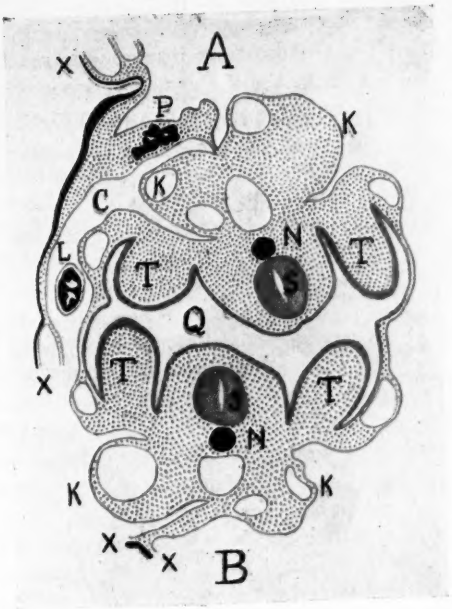

Fig. 8.

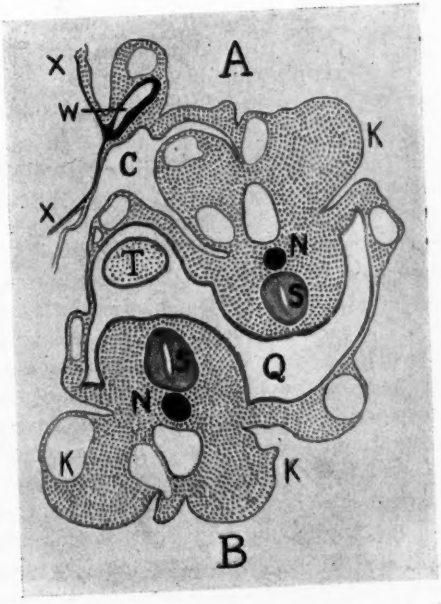

Fig. 9.

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was torn in removing the embryo from its amniotic sac (*XX* in fig. 5–9). Clearly below the level of reflection of this membrane mesodermal structures clothed by coelomic endothelium must everywhere project on the surface of the monster.

The caudal half of each twin contains a closed tubular prolongation of entoderm representing the hind-gut, the continuity of which with the external entodermal layer of twin *A* is seen in figs. 8 and 9. The caudal extremity of each hind-gut gives off an allantoic diverticulum of which only the torn pedicle is left, the remainder of this membrane having been lost in removal of the embryo from the egg.

The distribution of the respiratory rudiments is peculiar. Just as the fore-gut entoderm of each embryo has necessarily been unable to form a closed alimentary tube, so also there has been no formation of the median tubular respiratory diverticulum, and in each twin there are two widely separated caudally directed short lung buds arising from the external entodermal covering at points representing the true ventral wall of the unclosed fore-gut. Two of these are seen at *J* in figs. 5 and 6.

The liver rudiments and their relations to the entodermal envelope and to the vascular systems are seen in figs. 5–7. The liver of member *B* lies entirely within the "body" of the dual embryo, which on this aspect (the reverse of fig. 2) is clothed by a continuous external sheath of entoderm. On the aspect viewed in fig. 2, however, we have seen that this sheath is incomplete at the hepatic level, so that the liver of member *A* with its coelomic covering is enabled to protrude externally. Associated with this eversion of the liver at the level of reflection of the attenuated splanchno-pleura is the herniation of the heart from the widely open coelomic cavity at a level caudal to the liver itself (compare fig. 2 with figs. 5–7).

Each of the pancreatic rudiments *P* is situated normally in the dorsal mesentery (figs. 7 and 8).

(2) *Ectodermal epithelia*

As is evident in figs. 4–9, the cutaneous ectoderm lines a central cavity *Q* which is really an amniotic gulf extending cranially from between the divergent tails of the monster. The backs of the two-member embryos lie opposite one another with this amniotic gulf between, and at the lateral margins of each member the somatopleure turns dorsally to meet and become continuous with that of the other member. This purely lateral fusion of the dorsally opposed embryos with the resulting enclosure of part of the amniotic space extends nearly to the most cranial part of the monster, where, however, total fusion of the two heads is present and the amniotic gulf comes to a blind end. The topography of this region has become very distorted and complex as the result of growth of the brains and other cephalic structures. The enlarging fused head, being limited cranially by the externally investing layer of entoderm, has expanded caudally into and distended the upper blind end of the space *Q*

between the two embryos. Thus the most cranial limit of this space is a circular sulcus or fornix formed where the trunk entoderm is reflected caudally on to the central caudally projecting cephalic mass. As a result of the expansion of this mass the gill clefts have become dilated, and, as already noted, the four eyes of the enlarging heads partially protrude through the four first clefts. We can now appreciate the reason for the early macroscopic observation that the eyes of the monster, unlike those of the normal embryos, lacked detail and were indefinite in outline. This was because the eyes were not truly superficial as under normal conditions, but were partially obscured by the surrounding lips of the gill clefts which formed, as it were, their embrasures. It is as if fused twins with a common gut and a large solid head were to be turned inside out and were then to peer out on the world through their own distended gill clefts. Indeed this simile, though only a simile, is very helpful in enabling one to visualise the general structure of the malformation under discussion.

All structures normally derived from the cranial cutaneous ectoderm are represented in the fused head region of the monster. Olfactory pits ***F***, optic lenses, Rathke's pouches, and otic vesicles ***O*** are present (fig. 3). The distorted Rathke's pouches deserve special comment. Each commences in the cranio-ectodermal fornix already described, and extends upwards between the entoderm externally and the fused brain internally. As already remarked, there is a slit-like communication between each pouch and the adjacent entoderm, indicative of the disappearance of the bucco-pharyngeal membrane. The fundal extremities of the two pouches extend almost to the vertex of the monster, where they lie close together, but not in contact, against the upturned floor of the fused brain.

Four pairs of limb buds are present, the upper ones, ***T***, appearing as rounded projections at the sides of the dorsa of the embryos about the centre of the trunk region (figs. 7–9), while the lower pairs are situated as in the normal embryos in the coiled caudal region.

(3) *Neural ectoderm*

The central nervous system consists of a fused brain and two separate brain stems and spinal cords. The latter are normal, but the cerebral region requires special description. Figs. 10 and 11, made from plaster of Paris reconstructions , explain the peculiar topography of the parts. Clearly the most cranial part, ***H***, of the cerebral rudiment is really the floor or ventral aspect of the fused brain, for it is on this aspect that the entoderm and the two Rathke's pouches lie. Conversely the most caudal or dependent part of the central cephalic mass, ***P***, is the true cerebral vertex. In this region some irregularities in the walls of the neural tube (seen in fig. 4) probably represent the pineal rudiments. The brains are thus inverted in position.

Fusion of the two cerebral rudiments has occurred just anterior to their diencephalic regions, for the fused central portion, ***HP***, bears the two pairs of eyes, and the position of the two Rathke's pouches already described indicates

that, on the upturned cerebral base *H*, fusion has been in the hypophyseal region. No separate hypophyses are present, and it is probable that these structures are fused together and are represented in the central cranially projecting diencephalic base *H*.

In each hind-brain region there is an abnormal acute dorsal flexion, the metencephalon being doubled back on itself and presenting an acute ventral convexity *X*, projecting cranially and nearly level with the diencephalic base *H*.

The optic vesicles are of normal structure and are attached to the fused diencephalon by hollow optic stalks situated nearly transversely tangential to the upper (or ventral) aspects of the vesicles.

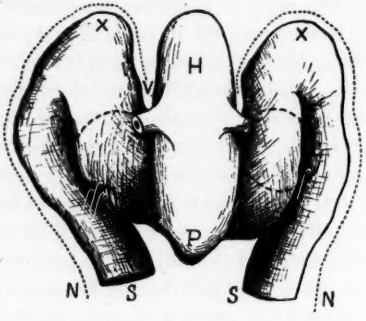


Fig. 10.

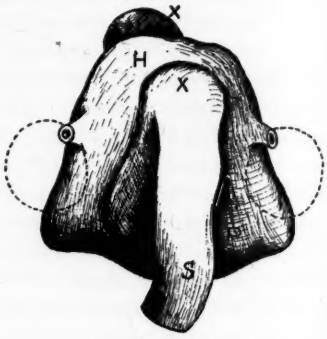


Fig. 11.

Fig. 10. Drawing of reconstructed model of the brains viewed from the same aspect as fig. 2. *SS*, spinal cords; *XX*, convexities on ventral walls of hind brain produced by acute dorsal flexure of neural tubes; *HP*, fused diencephalic parts of the two members, *H*, being the basal or hypophyseal region and *P* being the vertical or pineal region. Dotted lines *NN* indicate position of notochords, the cranial ends of which terminate in the acute angles *V*. Optic vesicles denoted by coarse dotted lines. × 20.

Fig. 11. View of brains at right angles to fig. 10. × 20.

(4) *The notochords*

In all sections the two notochords lie just beneath the external entoderm and on the ventral or outer aspects of the neural tubes. In the head region the position of the notochords is indicated by *NN* in fig. 10. Each follows the flexures of the brain stem, extending over the cranial convexity, *X*, of the hind-brain, and then turning caudally to terminate in the acute angle, *V*, between the brain stem and the cranially projecting base of the diencephelon *H*. These relationships serve to verify what has been stated above regarding the identity of the various regions of the distorted brain.

(5) *Mesodermal structures*

As already noted, where the entoderm ceases to clothe the monster externally and is reflected off the organism in an attenuated splanchopleuric membrane *XX*, coelomic mesothelium must necessarily appear on the exterior of

the more caudal parts of the monster. Thus in figs. 5–9 we see at various levels the projection of the heart, liver, kidneys, genital ridges and other structures from the open coelom. As we have seen, this presentation of mesodermal structures on the surface of the monster is greater on the aspect viewed in fig. 2 than on the reverse side. The normal coiled tails possess a complete ectodermal investment, so that here no mesodermal structures appear externally.

The various viscera and their mesenteries have largely developed normally, though necessarily distorted by the peculiar configuration of the parts. There is inter-communication and partial fusion of parts of the two vascular systems, the details of which, however, are beyond the scope of this paper.

Finally we may note why the mesodermic segments and the limb buds were obscured on macroscopic examination of the monster. It will be clear from figs. 7–9 that the somites and limbs of the embryos were not superficially situated as in normal embryos, but were hidden from external inspection by more external tissues. The true "exterior" from which segmentation and limb buds would be visible is the inter-embryonic amniotic gulf *Q*.

III. INTERPRETATION OF THE MALFORMATION

I was for long at a loss for an embryological explanation of the unique structure of this twin monster, but after repeated study of all features of the specimens, I believe the following interpretation to indicate the probable mode of origin.

From the foregoing description it is clear that, while two complete individuals are represented in the monster, the alimentary entoderms of the two members are continuous with one another. It must be assumed, therefore, that the early stage of development of the twin embryonic area was as depicted in fig. 12, I. Two individual ectodermal laminae with contiguous cephalic ends overlay a common entodermal sheet representing the twin entoderms in cephalic continuity with each other. With the growth of the embryos and the enlargement of the amniotic cavity, the egg, which was only of normal size, became too small to accommodate the two members in the position represented in fig. 12, I. Mechanical flexion of the embryos therefore occurred in the hind-brain region, the tails becoming approximated to each other, and the twins thus being folded back to back, as depicted in fig. 12, II. In this way was produced the cerebral inversion and the acute dorsal flexures of the hind-brains seen in fig. 10. Further growth of the embryos and absorption of yolk was accompanied by the normal "mushrooming" up of the entire monster into the enlarging amniotic sac. Thus the condition represented in fig. 12, III, essentially identical with the specimen described, was attained. In removing the monster from the egg, the delicate amnio-splanchno-pleuric membrane would be ruptured at *XX*, its attachment to the body of the monster, and the allantoic diverticula also would be torn in the same region.

Now cross-sections *P* and *Q* of the condition represented in fig. 12, II, would appear as in fig. 13, and it will be evident that, if the external attenuated

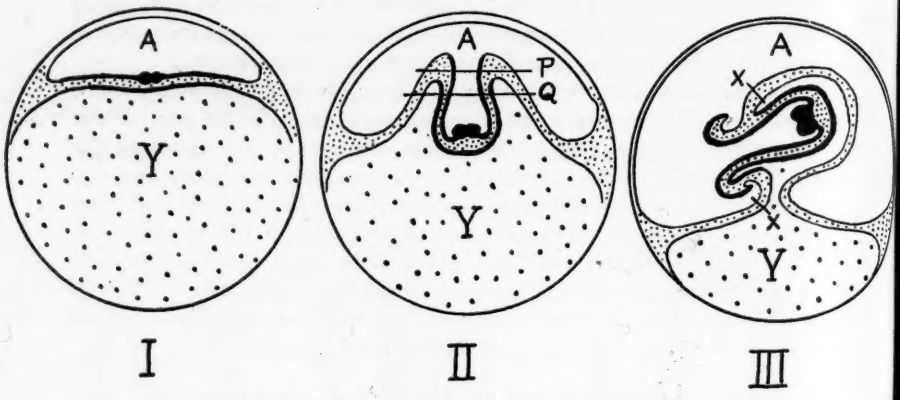


Fig. 12. Diagrams of hypothetical mode of formation of the monster. *A*, amniotic cavity; *Y*, yolk; *XX*, points of detachment of amnio-meso-entodermal membrane. Coelomic split not represented in dotted mesoderm, and nervous system not represented as distinct from ectoderm.

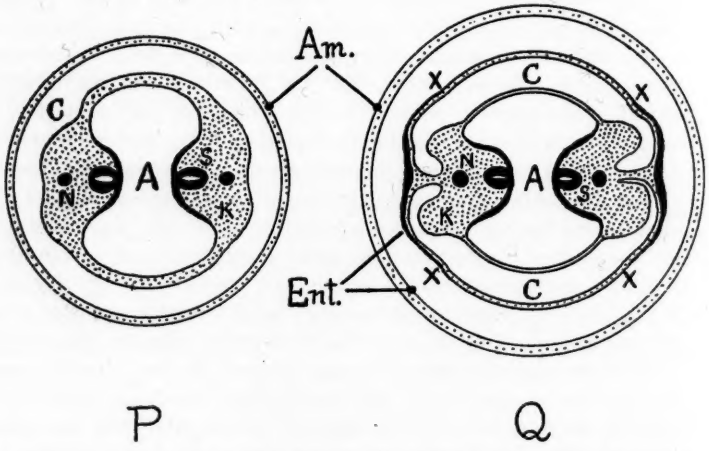


Fig. 13. Hypothetical sections *P* and *Q* of fig. 12. *A*, gulf of amniotic cavity between embryos; *Am.*, amniotic membrane; *C*, coelom; *Ent.*, entoderm; *XX*, points of tearing of attenuated splanchnopleure. Mesoderm dotted.

layers be torn away at *XX*, these hypothetical cross-sections are essentially identical with the actual cross-sections shown in figs. 8 and 9.

I conclude, therefore, that the malformation described arose by mechanical folding back to back of cephalically fused twins, resulting in (*a*) inclusion of an interembryonic portion of the amniotic space between the opposed dorsa, (*b*) complete eversion of the entoderm which thus clothes the monster externally and has been unable to produce a closed alimentary canal except in the caudal regions, (*c*) dorsal continuity of the somatopleures of the two members. I have been unable to discover in the literature any record of a condition at all resembling that described.

ACKNOWLEDGMENT

I am indebted to Prof. Wood Jones of the Melbourne University for examining the specimen and confirming my conclusions regarding its mode of formation.

THE INTRAGLANDULAR CLEFT OF THE PITUITARY BODY AND ITS CONNECTIONS

By JOHN BRANDER, M.D.

Notwithstanding the vast amount of literature and study devoted to the pituitary body and its functions in recent years, it is manifest that there remain many unsolved problems, some of which present anomalies which cannot readily be reconciled. The object of this communication is to direct attention to certain anatomical features which do not seem to have obtained the recognition which they deserve. They came to light several years ago in the course of investigations directed to a totally different object. If the observations then made receive confirmation they would appear to open up a further line of study and to offer a possible explanation of some of the findings which so far have remained inexplicable. It is not proposed now to refer in detail to the pathological aspects, although there is good reason to believe that the interpretation of the pituitary which will be put forward is in harmony with conditions which have been encountered in a variety of morbid states. Study has been devoted mainly to human material, and in any descriptions given reference is made to the human pituitary unless otherwise specified.

Whilst serial sections of the entire organ were being cut in a sagittal plane it was not unusual to encounter in the capsule breaches which receive little or no attention in the text-books. It seemed worth while pursuing the subject further, more careful methods for the removal and preparation of the pituitary were adopted, and in some instances sections were cut of the organ still lying in the decalcified sella turcica. Subsequently several examples were cut in different planes.

The findings which resulted may be briefly summarised as follows:

(1) Between the capsule of the pituitary and the periosteum lining the sella turcica, or, alternatively, between two layers of the dura mater of this region, there is a relatively large blood space which almost completely envelops the organ and communicates at several points with the blood supply of both anterior and posterior lobes above, and with the marrow of the sphenoid below.

(2) It appears that the lower end of the intraglandular cleft remains patent and in connection with the blood space just referred to, thus leaving a possible course to be followed by the colloid within the cleft when passing to the general circulation.

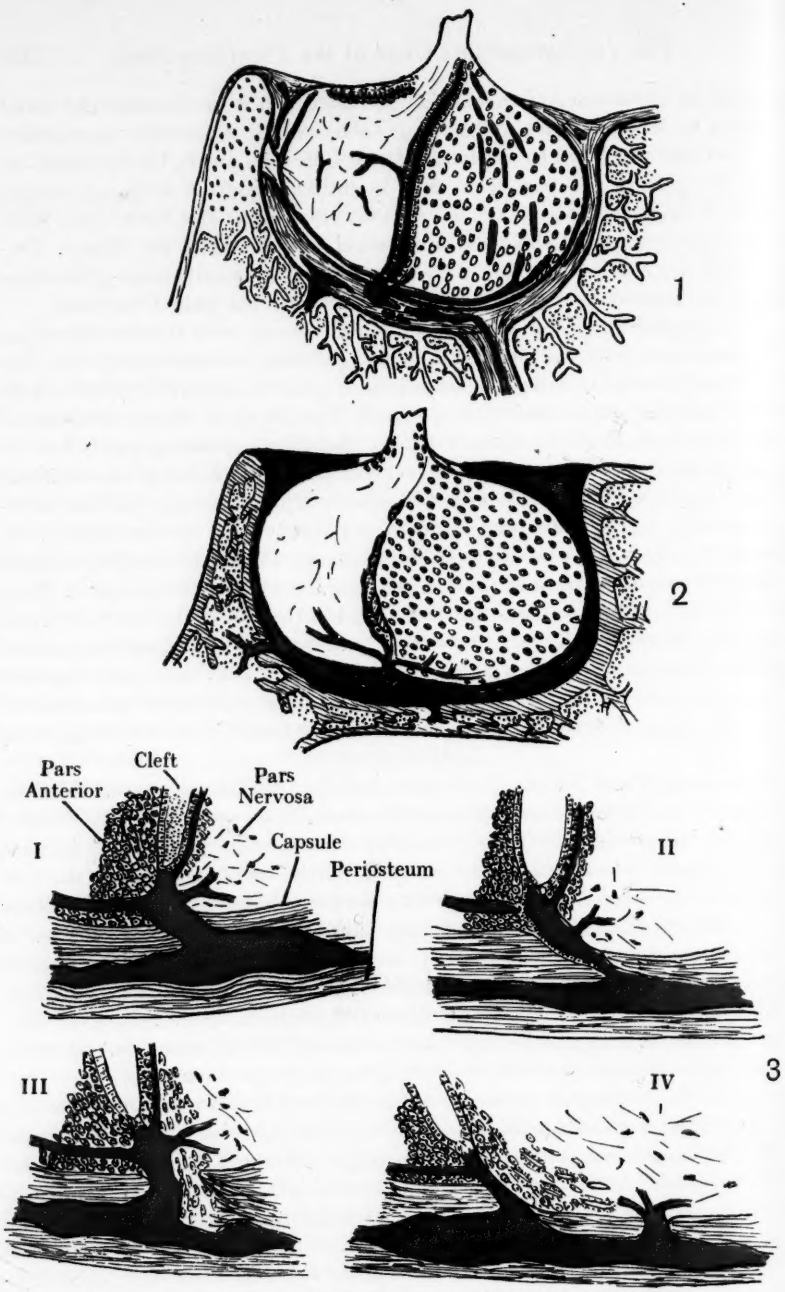
These two features may now be considered in greater detail. The peri-hypophysial blood space has probably the same mode of development as is recognised in the case of the other intracranial venous sinuses. It is generally

divided by trabeculae and indefinitely loculated, but it can be shown by serial sections to be continuous throughout its entire extent. A deceptive appearance is sometimes presented by sections in the middle line, due to the fact that the posterior pole of the pars nervosa is in intimate contact with and closely adherent to the bone over a small circumscribed area. The blood space may appear to terminate at this point, but lateral sections expose the fallacy. Presumably this area corresponds to the point where, at an early stage of development, the nervous portion first makes contact with the wall of the sella.

At its upper limits the space communicates freely with the circular sinus. Laterally it is quite distinct from, though possibly communicating with, the cavernous sinus. Its lining of flat epithelium is continuous with that of vessels in the pituitary and within the sphenoid. The degree to which the space is distended with blood varies greatly, the variation depending partly on the vascularity of the surrounding parts and partly on local pathological conditions when these are present. At birth the capsule of the pituitary and the periosteum lining the sella are relatively, and perhaps actually, thicker than in the adult. The blood space is correspondingly smaller. A variable number of blood channels can often be seen passing through the capsule to the space. Their occurrence is erratic over the anterior lobe, but they are rather more constant over the posterior lobe, being found most frequently (1) a short distance behind the intraglandular cleft, (2) at the posterior pole, (3) at the upper posterior extremity, where blood channels may pass into the space or directly into the circular sinus. Coronal sections sometimes show lateral communications with the space.

Communications between the space and the sphenoid appear to be more numerous in the young child than in the adult. In all probability this circumstance is connected with the development of the sphenoidal air sinus. At birth a substantial vessel thickly sheathed with fibrous tissue passes from the substance of the sphenoid through a foramen in the anterior half of the floor of the sella. Within the sella it turns sharply backwards to the line of junction of anterior and posterior lobes. That is to say, it follows the course said to be taken by the cellular cord derived from Rathke's pouch when its upper attachment is displaced backwards by proliferation of the anterior lobe. This vessel later on loses its identity and becomes part of the large blood space, but its origin and termination are not entirely obliterated (text-figs. 1 and 2).

It will be found that extension of the air sinus backwards under the sella is associated with backward displacement of the vascular communications of the blood space to a point or points where the marrow of the sphenoid remains relatively abundant. In a proportion of cases in the adult human subject there persists a comparatively large foramen in the anterior portion of the floor of the sella. Through this there passes the vessel noted in the foetus. In these cases the sphenoidal sinus lies anteriorly and is relatively little developed. This foramen with the same significance is constant and persistent in cattle. When the sphenoidal sinus is well developed and extends along the entire under sur-



Text-fig. 1. Diagrammatic sagittal section of full-time foetal pituitary.
Text-fig. 2. Diagrammatic sagittal section of adult human pituitary.
Text-fig. 3. Schematic representation of vascular communications at inferior extremity of cleft. (See text.)

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face of the sella or even, rarely, for a distance upwards in the posterior wall, the chief vascular connections will be found behind this. In the latter event blood from the space passes through foramina in the bone to communicate with the large vascular space which lies over the posterior aspect of the basisphenoid.

It is difficult to form a definite opinion as to the direction of the blood flow in the space and in the various vessels associated with it. On the whole, it may be said that histological appearances indicate that blood passes from the pituitary to the space, and thence to the sphenoid and the circular sinus. There is, however, some evidence that in pathological states stasis or reversal of flow may occur.

Below the pituitary and usually close to the line of junction of pars anterior with pars posterior there run fairly constantly in a transverse direction two conspicuous vessels. They are present at birth, persist to old age, and do not appear to enter into the formation of the blood space at any stage. Their origin and termination cannot readily be determined, but they tend to follow the groove between the two main parts of the organ. In the lateral angles they lie amongst loose fibrous tissue in which may be seen many cells of a glandular character, resembling those of the pars intermedia. In relation to the vessels are small nerve bundles which occasionally can be seen to traverse the capsule and enter the gland at its lower surface. These vessels and nerves are usually closely attached to the capsule proper, but not infrequently run in the trabeculae which link the capsule with the periosteum lining the sella. In the latter event they are very easily lost in removing the organ for examination.

When sagittal sections are cut in series there will be seen in a few of the sections a gap in the capsule through which there invaginates into the pituitary an extension from the peri-hypophysial blood space. The resulting channel is variable in extent and direction, but always terminates in relation to the lower extremity of the intraglandular cleft. The lining of this channel is interesting and must be considered to have considerable developmental significance. As a rule the flat epithelium of the space is continued towards the upper limit of the channel, to the point where it meets the cleft or duct-like structures from the cleft. Occasionally, however, on the anterior wall the flat epithelium is replaced by definitely cubical cells which extend to the lower limits of the capsule. The posterior wall, in a majority of cases, is formed by a layer of endothelial cells lying directly over a portion of the pars nervosa. But not infrequently pars intermedia extends to and through the capsule, many of its cells lying scattered in the loose fibrous tissue forming the walls of the main blood space. It is difficult to explain the presence of cubical cells anteriorly and of pars intermedia posteriorly except by assuming that the channel through the capsule is in some developmental continuity with the original Rathke's pouch.

When a careful study is made of the channel described above and of its intracapsular relations several important points can be determined. In the first place, three definite vessels or groups of vessels open directly into the channel. These are (i) a vessel in pars anterior, running close to and parallel to the cap-

sule, which receives many branches from the centre of the gland, (ii) a vessel in the pars intermedia, which is vertical and straight in the foetus but becomes rather tortuous in the adult, (iii) one or more vessels from the centre and lower portion of the pars nervosa. The arrangement of vessels just described may be regarded as the standard type. If, however, the pars intermedia is well developed and extends to or for some distance behind the breach in the capsule, it may be found that the vessels from the intermedia and nervosa open directly into the main blood space at the posterior limit of the intermedia, a short distance behind the channel which extends to the intraglandular cleft. The conditions described can best be indicated in diagrams. Text-fig. 3 shows some of the possibilities: (i) shows the most usual arrangement; in (ii) the anterior boundary of the channel is lined by cubical epithelium; in (iii) the intermedia is carried through the capsule to the wall of the main blood space; in (iv) the intermedia extends for some distance back under the pars nervosa, and vessels from intermedia and nervosa open directly into the main blood space.

The channel through the capsule of the pituitary which is now under consideration can always be shown to come into direct relation with the lower extremity of the intraglandular cleft. It is also in alignment with the cleft. Although it has not been possible to obtain an individual section showing an uninterrupted communication between the cleft and the blood space, in serial sections the two structures can be shown to be in continuity. Sometimes a thin duct-like structure intervenes. Whilst the histological appearances alone seem to indicate that the lower end of the intraglandular cleft remains patent and in communication with the peri-hypophysial blood space, pathological findings lend very strong support to such a hypothesis.

It cannot be denied that the blood space below the pituitary as now described is subject to variations in size dependent upon intracranial pressure or, perhaps more accurately, upon the ratio of venous pressure to cerebro-spinal pressure. At certain stages in growth the pituitary is too large to be accommodated entirely within the sella and bulges upwards, rendering the diaphragma sellae tense. In that event the blood space is more or less obliterated for a time. Pathological obliteration of the space may result from causes which fall into two distinct groups: (i) increase in the solid contents of the sella, (ii) increased intracranial pressure operating from above the diaphragma. Without enlarging upon the many morbid conditions involved, it may be said that pronounced increase of intracranial pressure from any cause is liable to obliterate the space. In the presence of such pressure there occurs distension of the intraglandular cleft with colloid. At the same time colloid accumulates in tubular spaces of the pars intermedia and the pars tuberalis. In spite of current teaching there appears to be no valid histological evidence that these parts of the pituitary secrete otherwise than towards the intraglandular cleft. If the cleft be greatly distended there will be found in the posterior and postero-inferior regions of the pars anterior masses of colloid lying in irregular channels, some lined by flat epithelium and some apparently the result of disruption. The

colloid within these channels can be shown to be continuous with that which is within the intraglandular cleft. It must not be supposed that these channels are all of pathological origin, for, though inconspicuous, they are present in otherwise normal pituitaries from birth onwards. That the intraglandular cleft should become distended with colloid in consequence of pressure on the pituitary can only be explained as an obstruction phenomenon. It is impossible to suppose that pressure incites the lining of the cleft to a secretory function previously in abeyance, or that the glandular cells take on heightened activity. Why is colloid in quantity so often found in the postero-inferior part of pars anterior if there is no tendency for it to travel in that direction normally? In the blood space below the human pituitary there can sometimes be seen small amounts of colloid in association with blood. In the same site in the ox large masses of colloid have been encountered.

Sometimes when the intraglandular cleft is much distended the contained colloid can be seen to protrude directly from the cleft into the opening through the capsule to the blood space.

The microscopical appearances and pathological considerations described at once suggest that the buccal portion of the pituitary, in all its parts, may preserve its original character and be in fact an atypical tubulo-racemose gland. It is generally taught that the intraglandular cleft disappears in the adult human pituitary. This statement is misleading. It is true that the anterior and posterior walls fuse over considerable areas, leaving only what appear to be isolated cysts containing colloid. Serial sections will show that most, if not all, of these cysts retain communication with one another. Pathological retention of colloid at any age will re-establish a cleft readily visible to the unaided eye. Under the microscope the cleft thus distended is tortuous and crossed by trabeculae not seen in the large cleft of the infant.

The pars intermedia is often stated to be insignificant in the human adult. It is in fact extremely variable in extent and arrangement. Without entering upon consideration of the circumstances which influence such variability, it may be remarked that the main blood vessel associated with the intermedia always runs at or near the line of junction of the buccal and nervous portions of the organ. Consequently this vessel lies at a variable distance from the intraglandular cleft.

The fact that there is a relatively abundant vascular communication between all parts of the pituitary and the blood space which surrounds it has manifestly a possible bearing on the functional mechanisms of the organ. It is, of course, recognised that the passage of colloid by a duct directly into the blood stream introduces a novel physiological conception.

That such structures as have been described do in fact exist can only be proved by actual demonstration. Such demonstration, notwithstanding certain technical difficulties, is possible in so high a percentage of cases as to justify the belief that they are constantly present.

It may well be asked why were not these simple anatomical points, if they

do indeed exist, discovered long ago. Emphasis should be laid on the fact that all the present observations and conclusions were achieved in total ignorance of the work done on the same subject many years ago by Bela Haller, who, working at Heidelberg in 1895, reached very similar conclusions with regard to the pituitary throughout the animal kingdom (1). Summarising his views, Herring in 1908 wrote: "B. Haller states that in Mammals—as a type of which he takes the mouse—and in all other classes of vertebrates the anterior lobe of the pituitary and epithelial investment of the posterior lobe form a gland, the tubules of which open by a small median and ventral mouth into the space between the pia and dura mater. Haller believes that the pituitary in all vertebrates secretes directly into the subdural space (2)." Reference to Haller's original paper, especially to the engravings with which it is illustrated, leaves no doubt as to the close approximation of his results to those now recorded. So far as is known, these structures have not hitherto been demonstrated in the human pituitary. Edinger indeed denied that Haller's findings could be confirmed in the human subject.

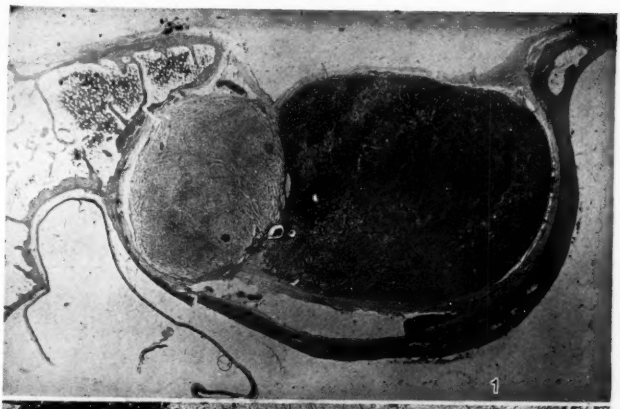
The blood space now described as being present below and around the pituitary was regarded by Haller as a lymph space in the dura covering the floor of the sella turcica. Herring remarked on the space and noted that whilst inconspicuous in some animals it is pronounced in the foetus of the ox, "where, at an earlier stage of development, it penetrates for some distance into the body of the sphenoid bone." In the adult ox the vascular space is continued as a stout vessel into the marrow of the sphenoid.

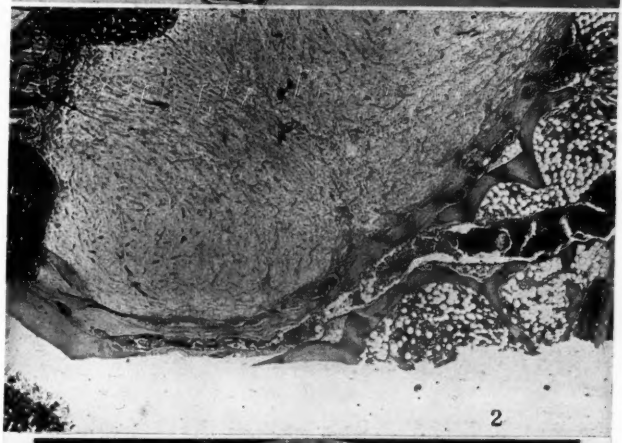
It may here be observed that, in the course of these investigations, no evidence was found of a subarachnoid space around the posterior lobe or around the pituitary as a whole. On the other hand there is enough evidence to justify a definite statement that at no stage of post-uterine life is such a space other than hypothetical.

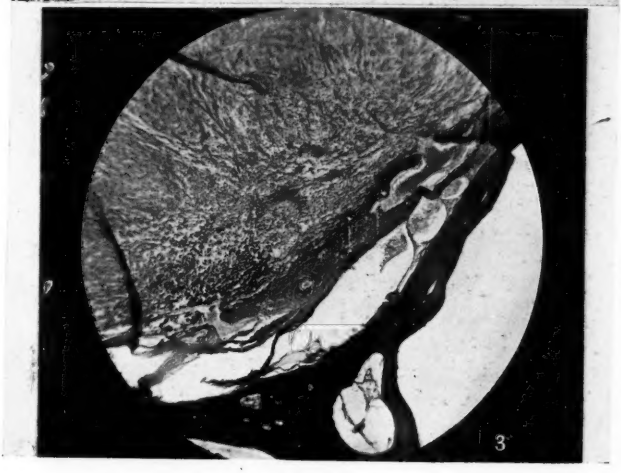
The various points at issue can perhaps most convincingly be illustrated by photomicrographs of selected sections. It must be recognised that in every process of fixation and embedding there occurs some degree of shrinkage and consequent distortion. The effect of this is naturally apt to be very noticeable in the case of specimens which contain in close association substances of such varied physical characters as pars anterior, pars nervosa, colloid, capsule, blood clot, periosteum and decalcified bone. Occasionally the distortion renders desired features more apparent. As a rule it is disadvantageous.

Seldom will a single section carry conviction, yet when studied as one of a series it may seem conclusive. In the choice of illustrations an attempt has been made to show as clearly as possible the structures described and also to indicate some of the diverse appearances which may be encountered.

In this communication attention has been confined to anatomical considerations. By common consent our present interpretation of the pituitary and its diseases is unsatisfactory. It is hardly necessary to emphasise the extent to which these data, if confirmed, may modify our interpretation of those accumu-







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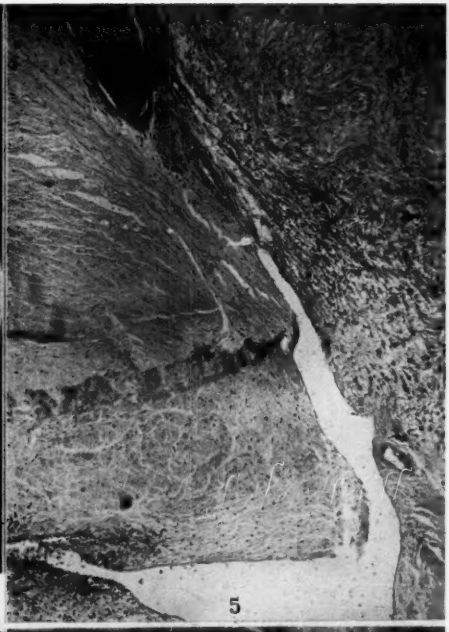

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lated records, physiological, biochemical, clinical and pathological, which to-day form a stupendous body of literature on the subject.

The writer gratefully acknowledges his indebtedness to Dr Geoffrey Clarke for the opportunity to carry out these investigations at Bexley Mental Hospital, and to Mr Charles Geary and Dr D. J. Reid for photomicrographs.

REFERENCES

(1) Haller, B. (1896). *Morphol. Jahrbuch*, Bd. xxv, S. 101.
(2) Herring, P. T. (1908). *Quart. Jour. Exper. Physiol.* vol. i.

DESCRIPTION OF PLATES

Plate I

Fig. 1. Sagittal section of an adult human pituitary lying within the sella turcica. The blood space below the pituitary is large and less subdivided than usual. A quantity of blood became dislodged during staining, but a wedge-shaped mass of blood can be seen below the pars anterior. In this case the sphenoidal sinus extended exceptionally far backwards.

Fig. 2. Sagittal section showing a large vascular connection between the blood space and the marrow of the sphenoid.

Fig. 3. Sagittal section of the lower portion of pars nervosa. Two relatively large vessels can be seen passing through the capsule to communicate with the blood space, one from the pars intermedia and the other from pars nervosa. The space is continuous although in this section the capsule and periosteum are blended between the vessels. The periosteum shrunk away from the bone during hardening.

Plate II

Fig. 4. Sagittal section at the inferior extremity of the intraglandular cleft. The blood space is seen between the periosteum and the capsule proper of the pituitary. From the space a channel passes through the capsule towards the cleft.

Fig. 5. Sagittal section of a young adult pituitary. The channel from the blood space is seen passing to, and apparently continuous with, duct-like structures at the inferior extremity of the intraglandular cleft. The indentation of the pars nervosa represents the termination of a vessel from the interior of that portion of the pituitary.

Fig. 6. A similar section from a full-time foetus. The channel through the capsule is in relation to the intraglandular cleft. The vessel from the pars nervosa is again indicated by an indentation.

Fig. 7. Sagittal section of the lower extremity of the intraglandular cleft in an adult human pituitary. The organ was unaffected by any gross disease but the cleft was considerably distended by colloid. Below the pituitary can be seen the blood space between the capsule and the periosteum which lined the sella. Colloid from the cleft protrudes directly into the blood space. During life the boundaries of the space were probably in apposition, the relations shown in the photograph being the result of shrinkage.

THE FUNCTION OF INTRA-ARTICULAR FIBROCARTILAGES, WITH SPECIAL REFERENCE TO THE KNEE AND INFERIOR RADIO-ULNAR JOINTS

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THE structures known as intra-articular fibrocartilages, or menisci, appear with remarkable constancy in the joints to which they are assigned by descriptive anatomy; not only in Man but also in other animals. They are therefore to be looked upon as integral parts of such joints, with definite parts to play in their workings. Nevertheless, they are undoubtedly often very variable as regards their size in a particular articulation: a disc stated to divide a cavity into two separate chambers is often found to be perforated to a great extent, and that without any apparent lessening of joint effectiveness. Further, it is a well-known fact that the removal of the internal meniscus of the knee is an operation not necessarily followed by discomfort of the patient in the use of the limb. There exists, then, the paradox that structures clearly of great importance for the normal working of a part can be removed (at least to the extent of one-half) without the production of ill effects. This paper is an attempt to present an account of the working of the intra-articular cartilages in terms of certain simple physical principles which are fundamental in joint mechanism, and which explain the effects of their absence as well.

PREVIOUS VIEWS

A survey of the literature available shows that attempted explanations of menisci[1] are morphological and functional. While the two are necessary for a complete understanding of any organ, they may be studied to a great extent independently. It is the second explanation which alone is treated of here. From the morphological side many different interpretations have been advanced: that they are structures *sui generis*; that they often represent elements of the skeletons of lower forms; and that they stand for tendons or ligaments taken into the synovial cavity (Bland Sutton, 1897). In their less ateleological moments, however, anatomists have paid some attention to possible reasons for the persistence of such remnants of a former glory. A summary may be given of the opinions expressed in the various English text-books. Either in the general or in the special sections of arthrology it is maintained that a

[1] The term "meniscus" is employed throughout this paper as a convenient term for an intra-articular fibrocartilage, without regard to the actual shape it may have in a particular instance.

meniscus (1) allows of dissimilar motions in the same joint; or, (2) abolishes the effects of incongruity between the male and female surfaces; or, (3) does both. Emphasis is usually laid upon the second of these rôles. It is assumed that the flexibility of the cartilage must obviously serve the purpose of fitting the surfaces together. This is also the opinion of Fick (1904, 1910), and it is seemingly still unquestioned in Germany (e.g. Broesike-Mair, 1930). (The latter textbook refers to the opinion of the brothers Weber that the menisci of the knee stand to the femur in the relation of wedges placed beneath a carriage wheel.) Goodsir's views on the matter will be taken up later; it is now pointed out that he adhered to the opinion that the disparity of the femoral and the tibial apposed surfaces was compensated by the elasticity of the interposed cartilages (Goodsir, 1858). Veterinary anatomists would appear to share these views. Sisson (1917) adheres to them explicitly, and adds that they help to prevent concussion. The last function had previously been ascribed to them by European workers.

Wood-Jones (1920) has criticised these explanations. He points out that all the Mammals (except monotremes) have a meniscus in the temporo-mandibular joint, even where only one kind of movement is permitted; and adduces the sterno-clavicular articulation as another example where distinct movements cannot be reasonably predicated of the two chambers formed by a meniscus. In regard to the congruence theory he says: "It is difficult to believe that Nature could not fashion the articulating bones to make an accurately fitting joint, and so adopted a plan of inserting washers to complete the adjustment"; and quotes Poirier's observation on the matter: "Cette explication de l'existence des fibrocartilages interarticulaires est loin de me satisfaire." It is a teleological answer to a teleological argument, and as such is quite pertinent to the discussion. As for the anti-concussional hypothesis, it is vitiated (in the writer's eyes) by the absence of menisci from such articulations as those of the talus, at least from the point of first importance.

FUNDAMENTAL PROBLEMS OF JOINT MECHANISM

The attempts which have hitherto been made to assess the working value of the menisci have this in common: that they assume them to be primarily related to the articular surfaces. These are large compared with the cartilages. It is here advanced as a principle of analysis, that in the interpretation of function parts of like dimensions[1] should be brought into association before the correlations with larger or smaller parts are sought for. There is one element of a diarthrosis which is of dimensions comparable to those of a meniscus—the synovial fluid. In practically all cases, the menisci divide the synovial fluid into roughly equal parts, each of which (by reason of the wedge-like form of the disc) is of comparable volume to the cartilage, that is, in the uninflamed joint. There will, then, be some positions in which the bones will be separated by a triple layer consisting of two films of synovia with a wedge of fibrocartilage

[1] The term "dimensions" may be taken either in the sense of absolute size, or with the meaning assigned to it by theoretical physics: length, mass, or time.

between. The first effect of an angular displacement of the laminar part of the meniscus will be an alteration of the relative thicknesses of the films: one will be increased and the other decreased in like proportion. Hence there will be a definite change in the character of the layers interposed between the bones; and it is of importance to determine the nature and effects of such an alteration, and the conditions in which it is likely to occur. It is convenient to deal with the second problem first, for it is merely another way of asking the question: in what circumstances are the bones entirely separated by the interposition of a synovial film?

In the construction of a diarthrosis two requirements have to be met. It must be possible for it to transmit weight through a temporarily stabilised couple, the components of which are displaceable, and it must permit of the free movement of the components on one another by the action of the related muscles (Walmsley, 1928). In a lecture delivered before the British Orthopaedic Association meeting at Belfast (in 1927) Prof. Walmsley summarised the main mechanical features of this class of joint. Following Goodsir, he developed the thesis that in the performance of the first function the joint surfaces are fully congruent and the articulation is in effect a synarthrosis, the synovial cavity being obliterated over the area of contact. In other positions they are incongruent. In the carrying out of the second function, therefore, a receiving (female) surface is continuously fitted by a male surface until the two are wholly congruent (that is, in movement towards the weight-bearing position: in movement from this position the surfaces will become more and more incongruent and they will separate to a greater degree). This had already been demonstrated at the knee (Bruce Young), at the atlanto-axial joints (Fick), and at the elbow (Hultkrantz). It was demonstrated by Walmsley at the hip, and has since been observed at the radio-ulnar joints (MacConaill, *infra*). The accessory articular mechanisms which bring about the synarthroidal disposition of diarthroses in the weight-carrying state are inactive in the function of movement. While in motion the epiphyses are not only rotated on each other but there is linear advancement of one area on another, so that the centre of rotation and the axis of rotation are always changing (Walmsley).

In the analysis just quoted attention was mainly directed to the terminal aspects of intra-articular relations. Further it was pointed out that no direct comparison was to be instituted between artificial and natural joints, in so far as the surfaces of the former tended to a maximal degree of congruence in all positions. There is no doubt that the usual constructions of the wheelwright and similar artisans do imply that the centres of rotation of the male surfaces are coincident with the centres of curvature (and, therefore, of rotation) of the corresponding female surfaces. The same is true of the models described by Fick for the elucidation of articular function (1910, *op. cit.*). It happens, however, that a body of knowledge has been accumulated by engineers concerning the actual internal state of fully lubricated joints in motion, from which important principles directly applicable to natural joints have been

derived. These principles follow directly from some simple properties of viscous fluids in contact with moving surfaces, and have been applied with conspicuous success to the solution of many difficulties of the practical engineer. Their importance for the present discussion lies in the leading place they assign to the shape of the lubricant film. They show that it has an active, not a passive rôle in the play of forces engendered by movement; they indicate the circumstances in which a special apparatus is necessary for its effective maintenance; and they explain why this apparatus may sometimes be removed without apparent result upon the working of the part.

FUGITIVE ELASTICITY AND ITS EFFECTS

In a classical paper on "The Theory of Lubrication," Osborne Reynolds (1886) showed that the most effective form of lubrication depends upon the formation of a liquid film between the moving surface and its bearing. When this articulation (as an anatomist may call it) is at rest the surfaces are in contact so far as their shapes permit, and no fluid intervenes between them. If the bearing be flooded with oil the condition of free movement is that it shall come to lie between the fixed and moving surfaces in such a way that it can transmit the thrust from one to the other. This entails the setting up of a pressure in the fluid between the surfaces, of a magnitude equal to the thrust of one towards the other, but opposed in direction to it. A film exhibiting such a counter-thrust is known as a pressure film. Prof. Reynolds found that a viscous fluid in such a system displays a characteristic reaction to motion. It develops an elasticity which may be regarded as breaking down at short intervals of time, and is consequently known as "fugitive elasticity." The effect of this reaction upon the movement of the surfaces depends upon their relative inclination. If they be parallel, the forces set up act to oppose the motion and to drag the fixed surface after the moving one. Moreover, the liquid develops no pressure to counter a thrust forcing the surfaces together. Hence, oil tends to be forced from between such surfaces rather than to be carried into the gap which separates them. Should one of the surfaces be inclined to the other at a small angle (fig. 1), then motion in the direction of the narrow end of the wedge-shaped space thus formed causes the development of a positive pressure in the fluid[1]. This pressure begins just beyond the inlet for fluid and attains its maximum on the outlet side of the centre line (*CC'*, fig. 1). The pressure has a twofold effect. It resists a thrust tending to force the surfaces together, and it forces lubricant through the outlet end of the gap. More lubricant will thus be drawn in through the inlet and a continuous supply assured so long as motion continues. The centre of pressure of the system lies between the area of maximum pressure and the line of centres. The load (weight-bearing) line passes through this point.

[1] In this and following descriptions the "horizontal" surface may be taken to be the moving one. Either surface may be in motion relative to the other, however: the important thing is that the two be inclined to each other in the direction of motion of the moving one. This motion would be parallel to the moving surface.

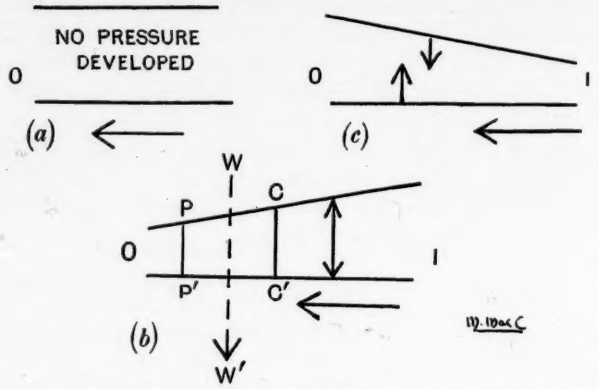

Fig. 1. The effect of the shape of a lubricant film on the forces set up within the liquid. (*a*) Surfaces parallel. (*b*) Surfaces inclined at a small angle. (*c*) Surfaces inclined at the same angle but movement in the opposite direction. *I*, inlet of lubricant. *O*, outlet of the same. The moving surface and direction of its motion are indicated by a horizontal arrow. Forces generated within the liquid are indicated by appropriate vertical arrows. In (*b*): *PP′*, line of greatest pressure; *CC′*, line of centres of surfaces; *WW′*, position assumed by load line (line of resultant of all pressures).

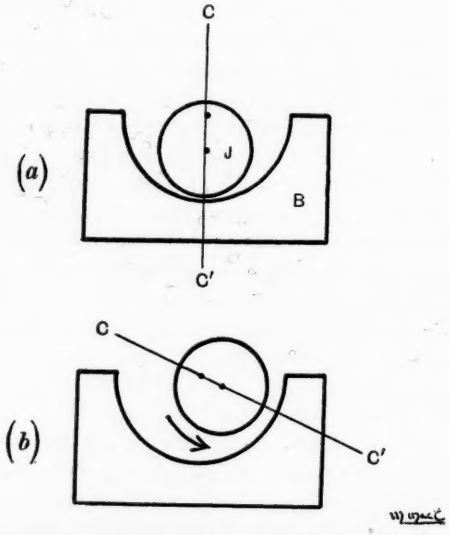

Fig. 2. Beauchamp Tower's experiment. (*a*) A journal (*J*) at rest relative to a brass (*B*) makes as close contact as possible with the brass, no lubricant intervening over the area of contact. (*b*) When the journal rotates relatively to the brass it sets itself eccentrically, forming a wedge-shaped space between it and the brass. Observe the direction of motion. *CC′*, line of centres of *B* and *J*.

Motion towards the wider end of the cuneiform space tends to draw the surfaces definitely together and to force lubricant from between them. The liquid is forced against the motion and lubrication is interfered with.

From these facts it follows that movement between plane, or approximately plane, elements of a joint requires the formation of a *wedge* of synovia if they are to transmit weight or other thrusts during movement. The wedge must be convergent in the direction of motion, so that fluid may be drawn continuously between the surfaces, and the film maintained. It need only be of small angle. In the case of curved surfaces, Reynolds showed that the male surface automatically sets itself at the correct angle to the female during rotation, that is, if the bearing be flooded. This entails a shifting of the line of centres, and therefore of the weight-receiving area of the female surface during motion. These points are illustrated in fig. 2. It will be observed that the centre of the male surface—its axis of rotation—travels in the direction of motion. This was first recorded by Beauchamp Tower, from whom Reynolds obtained much of his data. Engineers usually provide for complete rotations of curved parts, and there is generally a close approximation between the male and female curvatures. This one-to-one correspondence is not so necessary in the bearings with which the anatomist has to do. In fact, it can be reserved for one position, the synarthroidal one, in which the apposed elements of the surfaces must fit closely together. In other phases of movement an incongruence of the parts is a positive advantage in the light of what has just been recorded. It ensures the formation of the wedge of synovia without which smooth movements are impossible. This is the key to the meaning of the inequalities of curvature upon which Goodsir laid such stress. Further, the shift in the axis of rotation to which reference has been made above is seen to be a physical necessity, even in the case of a well-turned artificial joint. Both features of joint motion are expressions of the readjustments necessary to maintain the important cuneiform film between the acting parts.

So far it has been assumed that the forces set up in the film are sufficient to balance the forces tending to thrust the surfaces together. The force of fugitive elasticity varies with the coefficient of viscosity of the liquid and to some extent with the rate of motion in the bearing. Should the axial thrust exceed a critical value the resistance of the fluid breaks down, and the surfaces are forced into damaging contact. This is the condition known to motorists as "seizing." It is often thought that it has but one cause: insufficiency of oil; but it can occur in the presence of an abundance of it. In the latter circumstance it occurs at high speeds, at which the longitudinal thrust of the shaft is of great moment. The same relative disproportion of oil reaction to thrust appears where a rise of temperature lowers the viscosity of the medium: that is, it is more prone to occur with fluids of low viscosity. In the majority of cases the surfaces are parallel. It has been shown above that such a disposition is not favourable to the formation of a pressure film.

THE MICHELL THRUST PAD

The principles enunciated by Osborne Reynolds, although recognised to be fundamental, were not applied to practice until the beginning of the present century, when an Australian engineer, Mr A. G. M. Michell[1], made use of them in the invention of the thrust block which bears his name. It was designed to meet the strains imposed by modern speeds on the bearings of marine engines. An examination of its essentials will make clear the function afterwards to be assigned to the menisci.

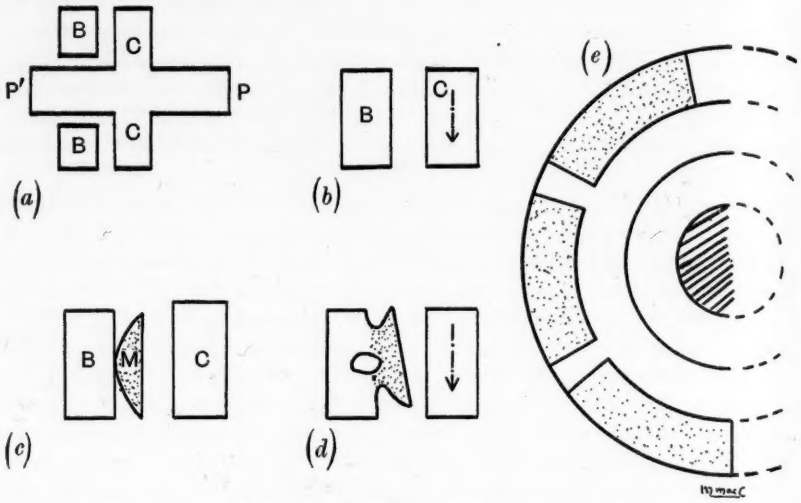

Fig. 3. (*a*) Section through a thrust bearing. *PP'*, propeller shaft. *C*, collar of metal shaft. *B*, thrust block. The thrust is in the direction *PP'*, the rotation through the plane of the paper. (*b*) Portions of block and collar to show that the motion is essentially between parallel surfaces. (*c*) Principle of the Michell thrust block. *M*, pivoted metal "pad," which can rock to-and-fro on the thrust block. (*d*) A Michell thrust pad (in this case connected to the block by deformable springs) sets itself automatically to the correct angle for smooth motion. (*e*) Surface view of a series of pads arranged to form a complete thrust bearing A horseshoe arrangement may be substituted.

A thrust bearing (fig. 3) consists of a block of metal pierced to allow passage to a rotating shaft. The reaction of the water on the propeller is transmitted to the thrust block by means of the metal collar, a film of oil separating the two while in motion. The bearing is flooded with oil, some of which is dragged between the block and collar by the movement of the latter. As both surfaces in apposition are plane they are also parallel. This entails a great liability of the film to break down at speeds well below the maximum attainable by the engine,

[1] A descendant of the Rev. John Michell who devised the method, and bequeathed to Henry Cavendish the apparatus, by which the latter determined the Constant of Gravitation.

and demands the provision of many such bearings to divide up the load. These add very much to the dead weight of the ship, an addition not repaid by the increase in efficiency. Mr Michell interposes a number of metal pads between block and collar. Each of these is sector-shaped in outline, with a plane collar face and a somewhat curved (convex) block face. The curvature of the block face permits the pad to rock through a small angle during motion; and accordingly it sets to the correct angle during movement of the collar, be this ahead or astern. Thus is ensured the formation and maintenance of the film wedge. It has been found that the connection between the pads and the block can be made by a double spring or some equally deformable continuous structure, since the deflection under the load has to be but a slight one. Such a flexible connection enables bearings to be simplified by constructing a number of pads integral with, but flexibly connected to, a common supporting member. A serious disadvantage is that the flexibility involves more or less risk of fracture of the flexible part, a danger which is overcome by giving flexibility to a portion of the pad itself (Michell, 1923).

These thrust bearings have proved very successful in practice. Their number can often be reduced to one, and other economies are effected by their use. In addition they allow of greater speeds, and are widely used to-day both in marine and land work in which high thrusts have to be met. Their purpose may be emphasised. They are employed where thrusts are to be carried by a film of lubricant during motions in which there is a considerable element of gliding, and in which a screw-like movement along the axis of thrust is discouraged.

THE MENISCI AS MICHELL PADS

A sector of a meniscus can be described as a wedge of elastic material, pivoted at its attached base, so that it is capable of some variation in its inclination to the articular surfaces with which it is in relation. The effect of such variation will be felt first by the synovial fluid: that is, the synovial film in contact with the articular surface will be made cuneiform to a greater or a less degree according to the amount of adjustment of the meniscus. Such angular deflections will be of small magnitude. Thus the sector is comparable in every respect to the most up-to-date type of Michell pad. The complete fibrocartilage is merely the integration of such elements, and may be of ring-, horseshoe-, or plate-like form. By reason of its flexibility, which is increased by the thinning of the central part, such a structure is easily affected by stresses in the synovia. The viscosity of this fluid is certainly not less than that of blood serum. Experiments are in progress regarding its actual value: but the table (see p. 218), which gives the viscosity of serum and of certain oils commonly used in film lubrication, will show that the requisite physical condition of the liquid for the application of Reynolds' laws to it is certainly present. The matter of the speed of the moving parts is dealt with below. (See further, p. 226.)

The synovial fluid, then, is one in which the phenomena of fugitive elasticity may be expected to appear, and the stresses set up in it by movements at the

joint will be of a corresponding nature. The part of the cartilage in relation to the weight-transmitting surface of the bone (at the moment) must so arrange itself that a wedge of lubricant, convergent in the direction of movement, is formed between it and that surface. Thus will be brought about the effective transmission of weight from the fixed to the moving surface through the fluid during motion, a transference which demands in certain cases a special apparatus that it be maintained without detriment to the continued separation of the surfaces. In a word, the menisci serve to increase the incongruence of joint surfaces rather than to decrease it. In this respect they are to be compared to the accessory cartilages of the hip and shoulder, although the function of the latter is not quite that of the menisci.

This statement of their function makes it clear where they are to be expected. They are likely to be found where a thrust is combined with gliding and rotation, if the consequent screw-like motion of the parts towards one another is not of service in the movement: that is, if it tend to put the bones into premature contact. If the surfaces be of large radii of curvature such contact is more likely to occur than if they be more rounded. A comparison of the carpo-metacarpal joint of the thumb and the sterno-clavicular joint will serve as an

Liquid		Coefficient of viscosity (c.g.s. units)	
Blood-serum	(32° C.)	1·9	(Burns, 1929)
Bayonne oil	20° C.	1·6	Stanton, 1923
Rape oil		0·90	
Sperm oil		0·34	

illustration. The view here put forward approaches that put forward by Parsons (1899–1900): it differs from it in being arrived at from first principles of mechanics and in defining more rigorously the locus of the structures concerned. Parsons' theory associated intra-articular cartilages with a combination of gliding and rotatory movements, laying special stress on the rotatory element. The present one rather reverses the emphasis, and takes cognizance of the fact that such combinations are present in practically every diarthrosis in the body, as Goodsir and his followers have justly maintained. They are present in the hip and shoulder. Here, however, they give a helical path to the moving point, at least in the former case. In so far as this tends to force the bones into premature contact, the well-rounded form of the heads of the long bones causes them to assume a favourable disposition to the female surfaces, as has been indicated above in the reference to Beauchamp Tower's experiment. It is possible, however, that the ligamentum teres may play the part of a meniscus in the hip joint, its consistency and disposition being not unfavourable for that function.

It should be added with respect to Parsons' theory, that the form he gave it was scientifically correct. It was derived from a purely morphological study and summarised all the facts observed by him in a manner consistent with the

physics of the time. Even yet, the principles of viscous-film lubrication are far from being widely known, except to certain classes of engineers; and the usual text-books of applied mechanics make but passing references to it. Those who are interested will find a full treatment in the article of Michell quoted above, and in Boswall (1928); while a simple presentation of its basal ideas is given by Edser (1926).

So far the treatment of the problem has been largely of an *a priori* nature with a view to establishing a reasonable hypothesis. It is not proposed to discuss all the joints which have intra-articular cartilages in what follows. The tibio-femoral and inferior radio-ulnar articulations have been selected as types for the demonstration and illustration of the lubricant function of the menisci. What is here proved for them holds for other joints; and in the ensuing discussion only certain of those others will be referred to as being of special interest.

KNEE JOINT

No detailed description of the form and attachments of the menisci is required. The points of importance for the elucidation of their function are: their wedge-like shape in section, the firm attachment of their cornua to the tibia, and the expansions of the vasti tendons which end upon them. A simple dissection is necessary to display the agreement of their relation to the bones with what has been premised above. The tibia is to be sawn through at the level of the upper part of its tuberosity. The cancellous tissue so exposed is to be removed carefully, and the deep surface of the compactum scraped clean. Holes should be drilled in the latter to emerge about the middle of the upper surface of each condyle. The openings are enlarged and the articular cartilage trimmed with scissors. The enlargement should be carried on until the lower surfaces of the menisci come into view. Care must be taken to keep their attachments intact, and it is well to be conservative of bone, so that a good estimate may be formed of the relations of cartilage and condyle in various phases of movement. A diagram of the views to be beheld through the "windows" is given in fig. 4. It was drawn by the aid of a camera lucida. In a like preparation the following facts may easily be observed:

Only in the position of full extension (with the proper rotation carried out) is there a complete congruence of the cartilages with the femur. In this, the "weight-carrying" position, the tibio-meniscal space is completely shut off from the femoro-meniscal; this applies to both condyles. In all other positions there is a wedge-shaped gap between bone and meniscus. These observations may be confirmed by an oil test. The bones are fixed in the weight-carrying position. The preparation is held so that the tibial windows look vertically upwards and the femur is below. Thin oil is poured into each opening. A pool is formed and remains for an indefinite time. In any other position whatsoever the oil passes from the tibio-meniscal to the femoro-meniscal chamber at once. Thin oil is chosen as it is a moderately viscous medium and its movements are easily observed. The experiment could be carried out with a watery fluid (albu-

men), but not in a specimen treated with oil; in this case surface-tension reactions between water and oil allow the liquid to slip through capillary spaces which would normally refuse passage to a viscous medium. The experiment shows that the menisci separate from the femur on the initiation of movement from the weight-bearing position in such a way that wedge-shaped spaces are formed between them and the bone, convergent in the direction of motion. The convergence will of course be noted for only one gap in each part of the double joint. If the corresponding part of the femur be marked it will be found to be that which is nearest to the tibia at the instant: it is the momentary weight-transmitting area. It has been shown by Michell and his school that the conditions of film lubrication are fulfilled if the weight-transmitting area be in contact with a convergent film—the disposition of the film elsewhere is not of importance. In extension, therefore, the anterior part of the cartilage is in

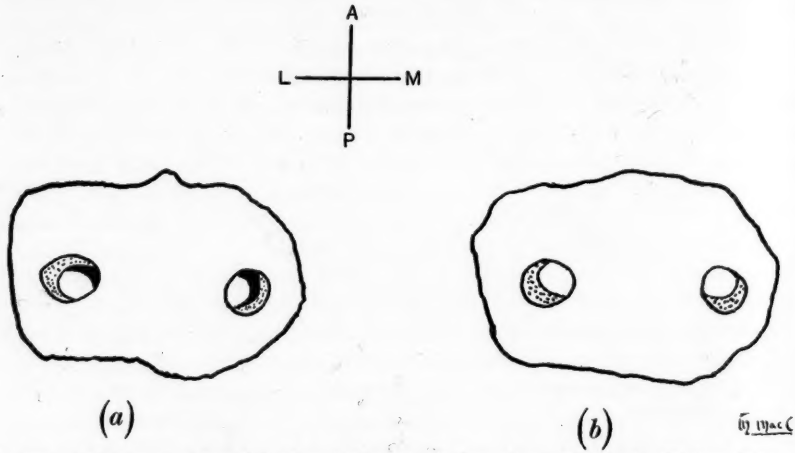

Fig. 4. Diagrams of knee joint from below, viewed through openings in the tibial condyles. The menisci are stippled. (*a*) in semi-flexion. (*b*) in full extension.

action as an aid to weight transmission; and fluid is carried from the patellar aspect of the femur to the under aspect, through the cuneiform gap. In flexion it is the posterior part, and in the rotatory movement, the lateral part that comes into effective play.

The meniscus, in short, behaves like a series of Michell pads welded into a generalised form, which is called for by the more complex movements of the animal joint. They are called for by the compound movement in which the gliding or sliding element is large in proportion to the rotatory. This does not permit of the self-adjustment which takes place between the components of a more purely spherical or hinge-like articulation. The "set" of the cartilage is largely automatic, and is conditioned by the forces generated within the fluid by movement. It should here be pointed out that the speed of the moving

surface is of importance in the formation of a pressure-film. It must have a minimal value of the order of 38 mm. (1·5 in.) per second. Experiments made by the writer on the living show that these velocity conditions are fulfilled at the knee: the least speed at which *smooth* motion is attainable having at least twice the critical value. The same, it may be added, holds for other joints. The function of the cartilage is assisted by its attachments to the tibia and to the vasti expansions. These bring about a tension of the meniscus and of its capsular attachment, so that it can take the fluid counter-thrust and pivot into the required angle. As movement continues, the cartilages move forwards independently of the tibia, for in a well-lubricated specimen it can be observed that their motion is markedly accelerated. In part this motion is

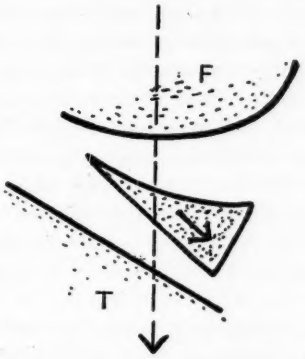

Fig. 5. A diagramt o illustrate the action of the menisci of the knee. *F*, an element of the femoral surface. *T*, an element of the tibial surface. The knee is moving from the semi-flexed to the extended position, and the motion of the cartilage relative to the tibia is indicated by the arrow within it. Femur and cartilage are, of course, moving in different directions. The direction of weight transmission is indicated by the broken arrow (load line). It will be observed to pass through the outlet (narrow) parts of the synovial wedges between the various moving surfaces.

due to reaction between the meniscus and the pressure-film of synovia, and it is a witness to the existence of the latter. Thus, as the flattened parts of the femur come into contact with the tibia, the cartilages are shot forwards with a twofold effect: the bones are permitted to come into direct relationship, and the posterior parts of the menisci are brought into contact with the femur, completing the separation of the upper and lower chambers of the joint (fig. 4). These effects are furthered by the opposite rotations of the cartilages described in the text-books. The posterior part of the cartilage is now "convergent" with respect to a femur about to undergo flexion. The whole mechanism is a very beautiful one, and the "wonder of the knee joint" is increased rather than diminished by a knowledge of the underlying simplicity of action. Fig. 5 illustrates in diagrammatic form the essential action of the cartilages as the femur

moves from the semi-flexed to the extended position, as in walking. The separation and angulation of the parts are, of course, exaggerated for the sake of clearness. The tilt of the cartilage relative to the tibia is to be specially noted. This tilt ensures the transmission of pressure from meniscus to tibia through the outlet end of the menisco-tibial synovial wedge.

With regard to the semilunar form of the menisci, it is of interest to note that horseshoe arrangements of Michell pads are quite commonly substituted for the annular disposition. Further, the larger size and more open form of the internal cartilage are precisely what are demanded by an application of the Michell principle, assuming that the most efficient lubrication be called for. Again, it has been shown that the total bearing surface of a Michell pad may be reduced to about half of the total possible area without inefficiency. The dimensions of the menisci of the knee, relative to the total effective articular surface at a given instant, are quite in keeping with this standard. Moreover, the occasional failure of the cartilages to perforate is no bar to a reasonably efficient action, since the elastic plate can function as a whole as it does, for example, in the temporo-mandibular joint (*infra*). In this "mass action" it is helped by the attachments of the vasti expansions. It is a corollary of the last affirmation that perforation of a cartilage which is normally complete is not necessarily disadvantageous, since the ring can function as a Michell pad system, provided that the reduction of surface does not appreciably exceed one-half. Rarely, in the writer's experience, does it go beyond that amount.

It remains, in connection with the knee joint, to account for the common absence of symptoms following the removal of a semilunar cartilage. The cartilages are accessories to lubrication, they might almost be called refinements on the part of Nature. Those in whom a violent effort provokes fracture of a meniscus—an accident previously explained to be conditioned by their flexibility—are persons of athletic habit. French surgeons, indeed, have noted a great increase of the number of such injuries since the introduction of "Anglo-Saxon" games—notably football (Jeanbrau, 1921). Loss of a meniscus leads to about 20 per cent. increase in friction. This amount is not usually reached in any but violent efforts—and the increased strain is likely to be unnoticed at a critical point in the game by a man in training. But it is recognised if ill-health supervene, and is remarked by those who have taken up exercise after a prolonged sedentary period, as the writer has verified. Many surgeons have recorded the incidence of osteo-arthritic changes in several of their cases, a result in keeping with the increased liability to premature contact of the articular surfaces of the bones on account of the removal of the counter-pressure set up by the synovial wedge.

INFERIOR RADIO-ULNAR JOINT

The inferior radio-ulnar joint is distinguished from the superior by the gliding character of the movement associated with it: a distinction which is emphasised by the wholly fleshy structure of its proper pronator. The triangular fibrocartilage is to be reckoned an accessory to this joint. The very full researches of Parsons (1899) have shown that the degree to which it is developed is positively correlated with the movement of supination, and that its best representative is to be found in Man. There are certain details of its structure and attachments which have to do with its function and demand preliminary notice; and some attention must also be given to both joints with regard to the position in which they assume the relationships characteristic of a synarthrosis, that is, in which they transmit weight directly from one component to the other.

The triangular cartilage follows the rule in that it is thicker circumferentially than centrally. The thinning is often stated to be central: but is more accurately described as centroidal—it is nearer the base than the apex of the triangle. When the thin part is perforated the greater part of the remainder bears a great resemblance to a semilunar cartilage. This likeness is heightened by the roundness of the angles of the cartilage proper. The disc is obliquely disposed between radius and ulna, sloping downwards and inwards from base to apex. Both surfaces are moderately concave, the greatest depression being, of course, at the spot already styled the "centroid" of the triangle.

SYNARTHROIDAL POSITION OF JOINTS OF FOREARM

Upper. The head of the radius is not circular but elliptical in outline. In a typical specimen the major axis is 2·4 cm. and the minor, 2·25 cm. This applies to the cartilage-encrusted bone. The curvature of the corresponding cavity of the ulna is equal to that at the extremity of the major axis of caput radii. Elsewhere the curvature of the male (radial) surface is less than that of the female (ulnar) surface. If a fresh joint preparation be examined from above, the annular and interosseous ligaments being intact, the synarthroidal position can be determined to be nearly half-way between pronation and supination. In this phase of rotation the head of the radius is most accurately adapted to the ulna, the long axis of its curvature being at right angles to the ulnar shaft, the shafts of the bones are most widely separated, and the interosseous membrane is at its greatest tension. The membrane is not quite taut: the significance of this fact will be discussed in a later paper dealing with the problems of weight carriage in the forearm. In any other position there is a small, but distinct, gap between the lesser sigmoid cavity and the head of the radius; this space is angular, and convergent in the direction of motion at the instant.

Lower. In many cases the triangular fibrocartilage is perforated: where this occurs a view of the relations of the head of the ulna to the radial articular surface is afforded. It can easily be determined that the head of the ulna is most closely applied to the radial concavity at the same phase of movement as

brings about maximum congruence of the elements of the upper joint. Each of the radio-ulnar joints is a virtual synarthrosis at the same instant, about midway between pronation and supination; and this is the definition of the weight-carrying position of the forearm.

THE MOVEMENTS OF THE FIBROCARTILAGE

A perforation of the triangular fibrocartilage is, in effect, a window for the inferior radio-ulnar joint similar to those made by dissection for the tibio-femoral. The opening is usually small, but it is large enough for the observation of the varying relationships of the ulnar head and cartilage. Only in one position does the rounded head of the ulna rest upon the disc in such a way as to occlude the opening—that position is the weight carrying one. In all others it is separated by a wedge-shaped space from the disc. The oil test is effective here, and demonstrates the complete congruence of bone and cartilage in the mid-prone stage of motion. If a little fluid be introduced into the joint cavity while it is in the fully supinated position it will be observed to be forced through that part of the wedge-shaped space which is convergent in the direction of motion; small air bubbles make its direction clearer. This shows the identity of the conditions in this joint with those in the knee, and with those set up by a Michell thrust pad.

The cartilage is, in fact, such a pad inserted between the hand and ulna. It enables weight to be transmitted from the hand to the ulna during movement of the radius across the forearm. This joint also illustrates the function of the congruence of the menisci with their proper bony opposites: it cuts off the flow of fluid and automatically brings the surfaces into direct play. It is to be noted that the path of the lower end of the radius is not a simple turn, but is a compound of sliding and rotation about the ulna of a somewhat helical kind.

COMPARATIVE ANATOMY

The comparative anatomy of these structures is quite well known as the result of Parsons' researches, and such an authority as Fick accepts them in their entirety. The writer has been able to verify them in such animals as were available, and the discussion of this part of the subject will be simplified by taking Parsons' accounts as standard descriptions. Reference need only be made to the knee, temporo-mandibular, and ankle joints to show the efficiency of the present explanation in satisfying the postulates laid down above.

Menisci are found in the knee joint as low down in the vertebrate scale as the amphibia (Bland Sutton). In Mammals the bat is unique in that it has not a trace of them. In this animal the joint is a pure ginglymus, rotation being prevented by a bony spur. In the English bat (*Vespertilio*) the tibial spur is very striking. In one form (*Plecotus*) a small amount of rotation is allowed, and delicate menisci are developed. More information is required on the landing movements of these creatures as the weight-carrying parts of their skeleton must differ both in site and development from those of non-flying Mammals.

In the lower monkeys the movement of rotation is better developed than in Man, and occurs even in the semi-flexed position. In such animals the semi-lunar cartilages are better marked, especially the outer. This is probably to be correlated with their swinging habits, which would tend to throw much strain on the knee, unless the latter were made more yielding in its movements than those of heavier types.

The temporo-mandibular joint is the classical example of the double-movement theory. It is not denied that such a double motion is found there, nor that the articular disc is a valuable accessory to it. But the forces which mould the flat articular eminence from infancy onward could equally well make one compatible with the condyle of the jaw, while still retaining its gliding movement. The existence of the disc in the carnivora furnishes an anatomical Voltaire with an excellent text against the "sufficient reason" of the theory. The difficulty disappears if the work of such discs in opposing a counter-pressure to that of the food be borne in mind. Although the trituration factor is absent from the carnivorous mandibular thrust, the tearing stress replaces it in importance; and it is necessary that the bearing surfaces be not prematurely forced together during the action of the carnassials. Intra-articular cartilages are not found in the jaw joints of the Monotremata, and they are likewise missing in certain of the Marsupalia (*Dasypus*, *Dasyurus*). Parsons has put forward the theory that the discus is a new mammalian acquisition, and thus accounts for its absence in these lowly forms. This view is not unacceptable: but further work is needed on the masticatory function of these animals before the absence of the structure can be held to be accounted for on functional grounds. It may be that such an explanation is unnecessary, as one covering positive instances can admit a phylogenetic account of origins.

The marsupials have a fibrocartilage between the astragulus and the fibula. It is to be correlated with the power of the fibula to rotate on its long axis (Parsons). The writer is inclined to associate its presence in the kangaroo with the strain thrown on the ankle at the termination of the leap; and in all these forms, the kangaroo and wallaby excepted, the fibula is very movable on the tibia and is liable to be drawn inwards by the tibio-fibular muscle.

DISCUSSION

The facts of human and comparative anatomy, so far as they have been recorded, seem to be in agreement with the thesis that the function of intra-articular cartilages is to maintain a convergent film of synovia between those bones the necessary movement of which is prejudicial to their continued separation during motion. This view of their working is in accord with the postulates of physics with regard to the transference of force between fully lubricated moving surfaces. It provides an explanation of their presence for the instances in which they occur. The only case of absence from a higher mammalian group is reconcilable with considerations of habit; and a *prima facie* case is recorded for an evolutionary reason of their non-appearance in

monotremes and certain marsupials. The congruence hypothesis has been shown experimentally, as well as theoretically, to be untenable. Goodsir recognised that the incongruence of articular surfaces of such joints as the knee must leave gaps between the bones. He deduced therefrom that the menisci and synovial pads had the common function of filling up the spaces. He definitely associated the cartilages with localities of pressure, making out that the other structures were found to occupy spaces free from strain. He was not aware of the laws of lubrication—they were not known in his time: nevertheless, in correlating the menisci with pressure, he made a good approximation to the truth. It may be said that the present essay is in some sort an extension of the principles he put forward regarding articular movements. The work of Prof. Parsons has already been referred to in the body of this paper—it is hoped, not unjustly. The difference between the points of view is largely one of emphasis. Finally, it may be said that an anti-concussional effect is not to be entirely ruled out. In many cases intra-articular cartilages must serve such a purpose: but only as a secondary result of their primary action.

SUMMARY

It is shown by reference to the theory of lubrication that intra-articular fibrocartilages are to be related primarily to the synovial fluid rather than to the articular surfaces of the bones. They act to bring about the formation of wedge-shaped films of synovia in relation to the weight transmitting parts of joints in movement. These wedges are narrowed in the direction of motion, and are necessary for weight transmission. They are to be found in joints where thrusts are likely to bring about a premature approximation of the joint surfaces. Preparations are described of knee and inferior radio-ulnar joints, which demonstrate the action of such cartilages. These also show that the cartilages are congruent with articular surfaces only in the "weight-carrying" position of the joints.

I wish to put on record my appreciation of the interest in, and encouragement of this work by Prof. Patten; and my thanks to Dr N. K. Adam, late Sorby Fellow of this University, for references to early papers on the theory of lubrication.

ADDENDUM

The experiments referred to on page 217 above have now been completed. They show that the viscosity of human synovial fluid is of the order of 10 C.G.S. units, at 20° C. This value is five times as great as that of blood-serum, but might fall as low as 7 units at body temperature. Accepting the latter value, it can be calculated that the thickness of the synovial film (knee) is of the order 50μ. Such a value is in full accordance with the principles set out above. Details of the experiments and of the calculations based thereon will be given in a future paper.

REFERENCES

Boswall (1928). *The Theory of Film Lubrication.* Longmans, London.

Broesike-Mair. *Repetitorium Anatomicum,* 2nd edn. Fischer, Leipzig.

Edser (1926). *General Physics for Students.* Macmillan.

Fick (1904). *Handbuch der Anatomie und Mechanik der Gelenke,* Erster Teil. Jena.

—— (1910). *Handbuch der Anatomie und Mechanik der Gelenke,* Zweiter Teil. Jena.

Goodsir (1858). "Mechanism of the knee joint," in *Anatomical Memoirs of John Goodsir,* vol. II, p. 231. Edinburgh, Adam and Charles Black. See also, "Anatomy of the knee joint," *op. cit.* pp. 220 ff., and "Curvatures and movements of the acting facets of articular surfaces," *op. cit.* pp. 246 ff.

Jeanbrau (1921). *Précis de Pathologie Chirurgicale,* Tome 4, 3me edn. Paris, Masson et Cie.

Michell (1923). "Viscosity and lubrication," in *The Mechanical Properties of Fluids* (a collective work). Blackie, London.

Parsons (1899). "The joints of Mammals compared with those of Man. I." *Journ. Anat. and Phys.* vol. XXXIV (N.S. vol. XIV), pt. 1.

—— (1900). "The joints of Mammals compared with those of Man. II." *Journ. Anat. and Phys.* vol. XXXIV (N.S. vol. XIV), pt. 3.

Reynolds (1886). "The theory of lubrication." *Phil. Trans.* p. 157.

Sisson (1917). *The Anatomy of the Domestic Animals.* W. B. Saunders Co., Philadelphia.

Bland Sutton (1897). *Ligaments, their Nature and Morphology.* Lewis, London.

Walmsley (1928). "The articular mechanism of the diarthroses." *Journ. Bone and Joint Surg.* vol. X, no. 1, pp. 40–45.

Wood-Jones (1920). *The Principles of Anatomy as Seen in the Hand.* Churchill, London.

THE TRACHEO-BRONCHIAL LYMPHATIC GLANDS

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The advance of surgery may call for the investigation of anatomical fields that previously appeared to be of little clinical value.

The present anatomical study was undertaken as part of an investigation of the possibility of irradiating the tracheo-bronchial lymphatic glands in bronchial carcinomata by the transtracheal and transbronchial implantation of Radon seeds.

This paper attempts to describe the shape, position and relations of the tracheo-bronchial lymphatic glands to be found in the mediastinum and hila of the lungs in an adult of over forty years of age.

A suitable terminology is suggested by which we may refer to the various members of this group, which should be of particular value to pathologists in giving an accurate description of glandular metastasis in the chest.

The *material* required for this investigation was obtained from the autopsy room in cases where there appeared to be no gross lesions in the lungs. The thoracic viscera were removed *en masse* by the usual post-mortem method, but special care was taken not to tear the lung tissue; a rubber tube was then tied into the trachea and the lungs distended with about 2000 c.c. of formalin solution.

The specimen was next put into a mould (made from the skeleton of a thorax) and surrounded by more formalin; thus the gross anatomical relations were maintained while the tissue was being fixed.

In seven cases the viscera were cut into serial macroscopic sections on a macrotome and in five others were dissected.

TERMINOLOGY

There is considerable variation in the terminology employed for these glands. Most authorities describe those on the sides of the trachea and in the angle between the two main bronchi, but no attempt is made to describe and name the various glands that lie in the angles of division of the larger bronchi.

Poirier, Cuneo and Delamere do not devote more than one page to the description of the "peri-tracheo-bronchial glands." They describe a right and left "pre-tracheo-bronchial" group of glands, an "inter-tracheo-bronchial" group and then they say: "The inter-bronchial glands occupy the angles of division of the larger bronchi. According to Cruvellier, glands may be found even in divisions of the fourth order."

Dickey describes the "bronchial glands" as follows:

(*a*) Superior tracheo-bronchial glands, a right and a left set, in the angles between the trachea and the right and left bronchus.

(*b*) Inferior tracheo-bronchial glands, between the two bronchi and in front of the oesophagus and aorta, nine or ten in number.

(*c*) Broncho-pulmonary glands lie in the angle which each smaller bronchus makes with the main stem of the bronchial tree.

(*d*) Pulmonary glands, lymphoid masses situated in the lung and the sub-pleural tissue.

Since all the tracheo-bronchial glands are in close contact with the bronchial tree and, broadly speaking, with the exception of the tracheal group, are wedged into the angles between the dividing bronchi, it would appear wisest to adhere to a classification that describes them in relation to this tree. Before we can proceed with the terminology of these glands, we must find a satisfactory nomenclature for the bronchi.

Let us consider for the moment that the bronchial tree divides in a dichotomous manner, and that there are two primary bronchi, four secondary, eight tertiary and so on in geometrical progression until the terminal bronchi are reached. Then those lymph glands between the primary divisions might be called "inter-primary" and for the others, a right and left inter-secondary, right and left upper inter-tertiary, etc.

The actual arrangement of the tree is, however, partly dichotomous and partly monopodic, and in addition is asymmetrical, but in spite of this, with a few modifications in the existing terminology of the bronchi, it is possible to have a classification of lymphatic glands based on this simple dichotomous scheme.

The *first* modification is to recognise that the left upper lobe bronchus divides almost immediately into two bronchi which are equivalent in their area of distribution to the upper (eparterial) and middle lobe bronchi on the right side. According to Narath, the left middle bronchus is phylogenetically the original, and the left upper (1st dorsal of Narath) has developed as a side branch from it. In addition it may be added that the arrangement of the blood vessels for this middle lobe area is the same on the two sides.

The *second* modification is to recognise that the first dorsal bronchus of the lower lobe (2nd dorsal of Narath) is of the order of a secondary bronchus rather than a tertiary branch of the lower secondary bronchus.

This bronchus arises from the dorsal surface of the "main stem" at a point exactly opposite the origin of the middle lobe bronchus on the right and just below the origin of the upper lobe bronchus on the left. Its external diameter is only 1 mm. less than that of the middle bronchus (Dickey). Its arterial supply arises from the descending branch of the pulmonary artery opposite the artery to the middle lobe, and its venous drainage is a large independent vessel that joins the inferior pulmonary vein just before it pierces the pericardium. Deve, who has devoted special attention to this lobe and calls it the "lobus posterior," points out that it is frequently separated off by a fissure

from the rest of the lower lobe, especially in children, where he states that it occurs in 50 per cent. of cases (counting bilateral cases as two), and in common with all accessory lobes is more frequent on the right. The dividing fissure of this dorsal lobe occurs as a continuation of the horizontal fissure, so that the lung is divided by a +-shaped cleft (see fig. 1).

The *third* modification is to substitute the word "ventral" for "middle" lobe or bronchus. It will be seen that this classification is based on the inner structural relations of the bronchi rather than on the morphology of the lungs.

To summarise our terminology for the bronchi: The trachea divides into two primary bronchi and on the *right* side the primary bronchus gives off from its right lateral wall a branch to the upper lobe, the upper secondary bronchus, and then continues as a main secondary bronchus which terminates by dividing into three secondary bronchi; a ventral branch to the ventral (middle) lobe, the ventral secondary bronchus; a dorsal branch to the apical portion of the lower lobe, the dorsal secondary bronchus; and the continuation of the main stem to the rest of the lower lobe, the lower secondary bronchus.

On the *left* side the primary bronchus is twice as long as on the right. It divides into two *main* secondary bronchi, upper and lower, which almost immediately break up into two more; thus the four secondary bronchi on the left side arise within a few millimetres of each other. The upper main bronchus divides into an upper secondary and a ventral secondary bronchus. The lower main bronchus gives off a dorsal secondary bronchus to the apical portion of the lower lobe and then continues to the rest of the lower lobe as the lower secondary bronchus. These secondary bronchi divide into tertiary, quaternary, etc.

THE TRACHEO-BRONCHIAL GLANDS

These glands form the "packing" that occupies the cracks and corners between the complex system of vascular and tracheo-bronchial tubes to be found in the mediastinum and hila of the two lungs. They may be classified as follows:

I. *Those in the mediastinum*

1. Right tracheal:
   - (*a*) Transverse.
   - (*b*) Vertical.
2. Left tracheal:
   - (*a*) Ascending.
   - (*b*) Central.
   - (*c*) Descending.
   - (*d*) Anterior.
3. Inter-primary:
   - (*a*) Superficial.
   - (*b*) Deep.

II. *Those in the hila of the lungs*

1. Right upper inter-secondary.
2. Left upper inter-secondary.
3. Right ventral inter-secondary.
4. Left ventral inter-secondary.
5. Right dorsal inter-secondary.
6. Left dorsal inter-secondary.
7. Right upper inter-tertiary.
8. Left upper inter-tertiary.

III. *Those in the lung fields*

1. Ventral inter-tertiary.
2. Dorsal inter-tertiary.
3. Lower inter-tertiary.
4. Inter-quaternary, etc.

The description that follows represents the average state found in the study of twelve cases (two-thirds of which were females) with an average height of sixty-five inches and age of sixty-five years.

1. *The right tracheal group* (*R.T.*) lies along the right antero-lateral surface of the trachea in the thorax. The group has a J-shape, having an expanded lower end that lies transversely and a vertical portion that runs up the trachea.

The transverse part (*T.R.T.*) consists of one large gland lying medially and two to three smaller ones lying laterally. They extend from the mid-line of the trachea onto the superior border of the right upper bronchus. The right pulmonary artery forms their lower boundary, which level also corresponds approximately to the internal bifurcation of the trachea (carina). The ascending branch of the right pulmonary artery separates the lateral glands of this group from the right upper inter-tertiary and in those cases where the glands are large, the transverse group are grooved by this artery and extend across in front of it to meet the inter-tertiary, so that the artery passes under an arcade of gland tissue as it crosses in front of the upper bronchus. In front of these glands is the ascending aorta and the superior vena cava. As the vena azygos arches forward to join the vena cava, it grooves the superior border of these glands.

The vertical part (*V.R.T.*) consists of one long gland below and six to eight smaller ones above. They extend from the upper border of the transverse part, in the mid-line of the trachea, upwards and backwards so as to lie on the right antero-lateral side of the trachea. Above they may appear to be continuous with the deep cervical chain in the neck (except that the latter have no black pigment), while in the other cases they end abruptly against the first part of the right subclavian artery. This vertical part is triangular in cross-section, on the medial side is the innominate artery and on the lateral the superior vena cava and behind this the parietal pleura and lung. The right vagus nerve runs

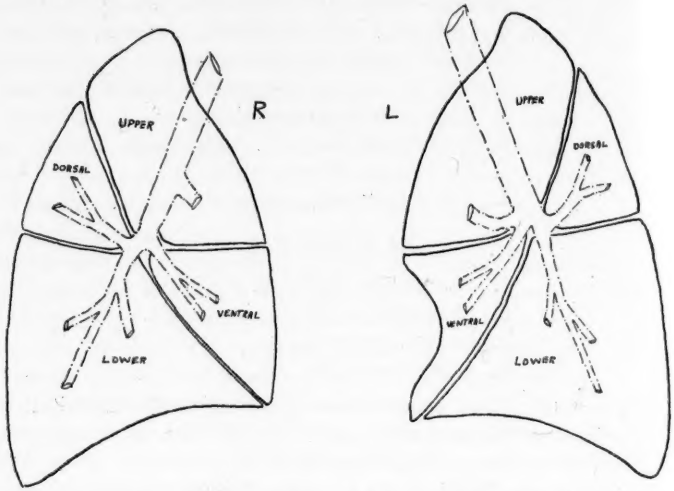

Fig. 1. Diagram showing the distribution of the four secondary bronchi on each side

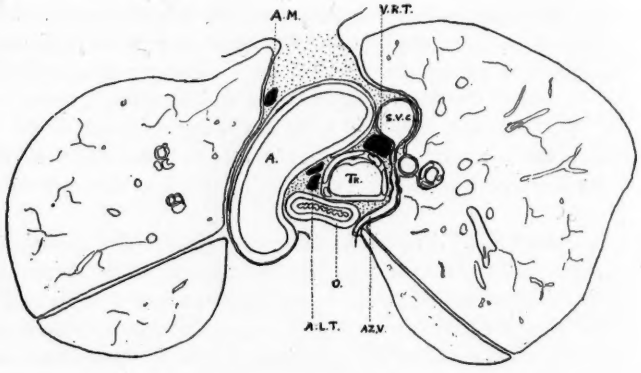

Fig. 2. Transverse section about 1 cm. above the level of the carina. The vertical part of the right tracheal glands (*V.R.T.*) and the ascending part of the left tracheal (*A.L.T.*) are shown.

The explanation of the letters used in this and the subsequent figures is found in a table at the end.

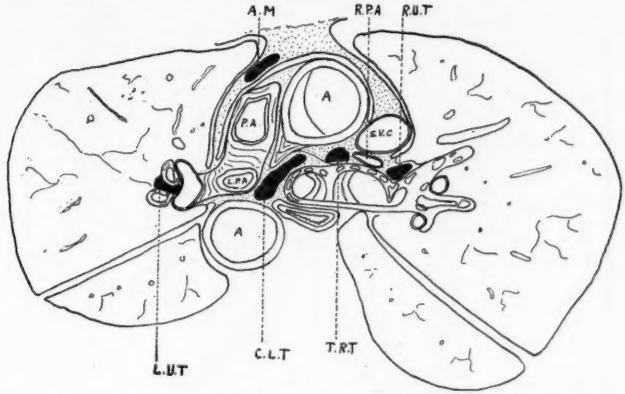

Fig. 3. Transverse section at the level of the carina, and just below the arch of the aorta. The central part of the left tracheal (*C.L.T.*) is seen between the pulmonary artery and the left primary bronchus. The ascending portion of the right pulmonary artery is seen between the transverse part of the right tracheal glands (*T.R.T.*) and the right upper inter-tertiary (*R.U.T.*). The anterior mediastinal (*A.M.*) and the left upper inter-tertiary (*L.U.T.*) are also shown.

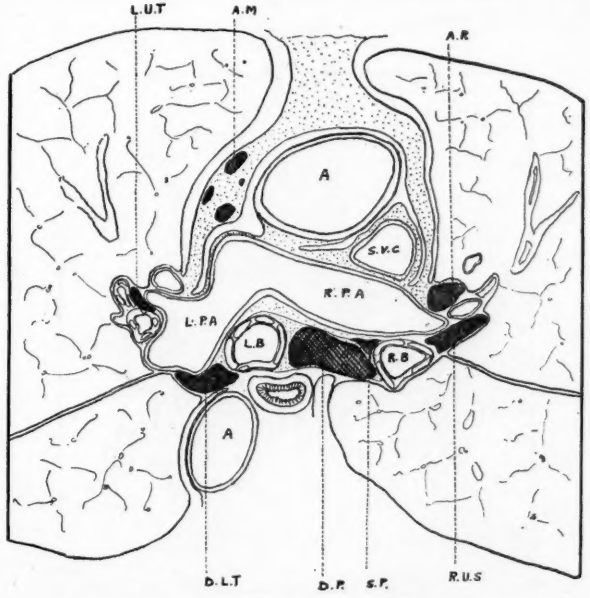

Fig. 4. Transverse section at the level of the bifurcation of the pulmonary artery. The deep and superficial parts of the inter-primary glands (*D.P.* and *S.P.*), the descending portion of the left tracheal (*D.L.T.*), and the right upper inter-secondary (*R.U.S.*) are shown. The anterior radicular gland (*A.R.*) is also shown in this section.

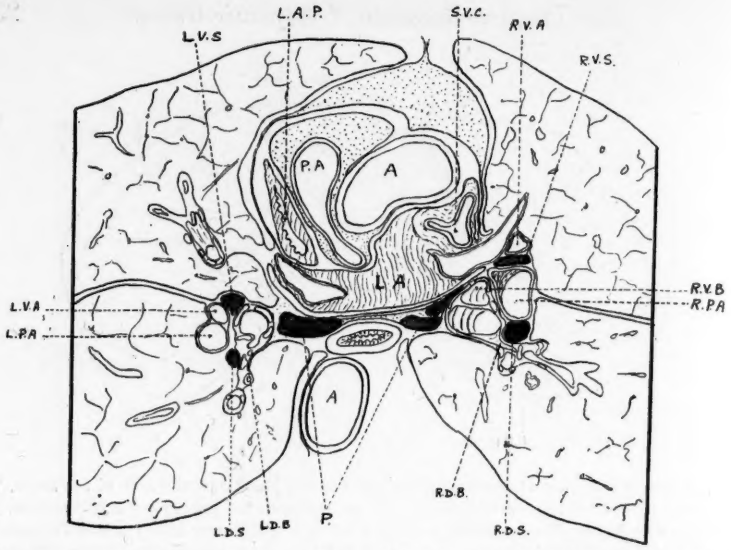

Fig. 5. Transverse section cutting across the top of the left auricle. The dorsal bronchus with the dorsal inter-secondary gland is shown on both sides (*L.D.S.* and *R.D.S.*). The ventral inter-secondary gland is also shown on both sides (*L.V.S.* and *R.V.S.*) and the inter-primary can still be seen in this section.

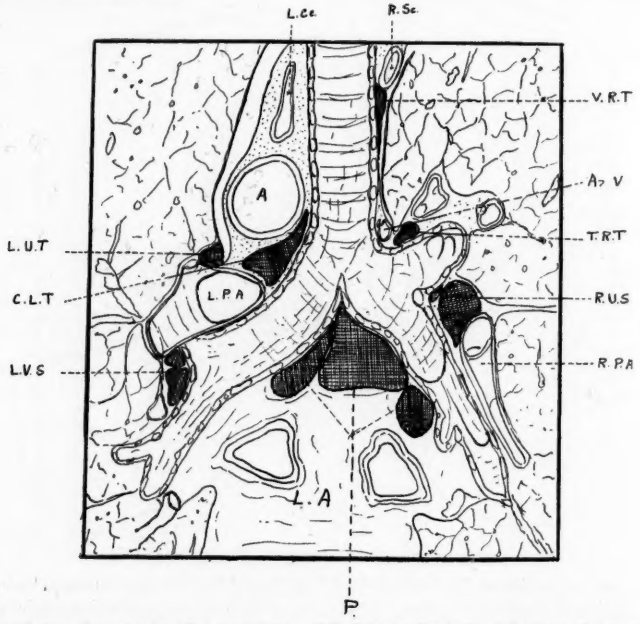

Fig. 6. Coronal section showing the central part of the left tracheal, the right upper inter-secondary, the left ventral inter-secondary and the inter-primary group.

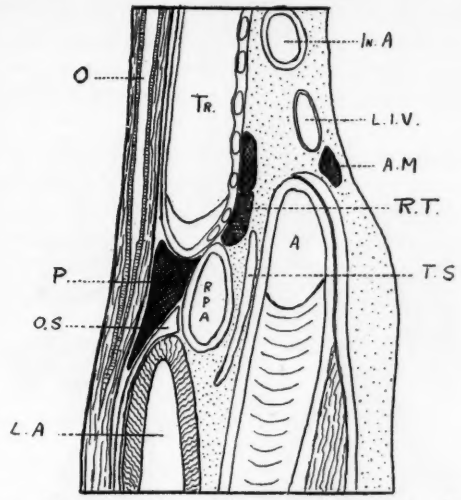

Fig. 7. Sagittal section of the mediastinum in approximately the mid line. Showing the lower end of the right tracheal group and the inter-primary.

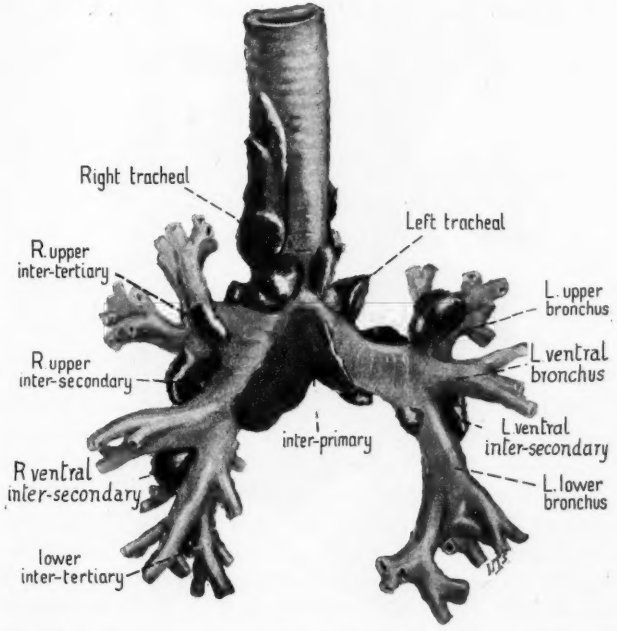

Fig. 8. A dissected specimen, anterior view, showing the typical form of the bronchial tree and the glands.

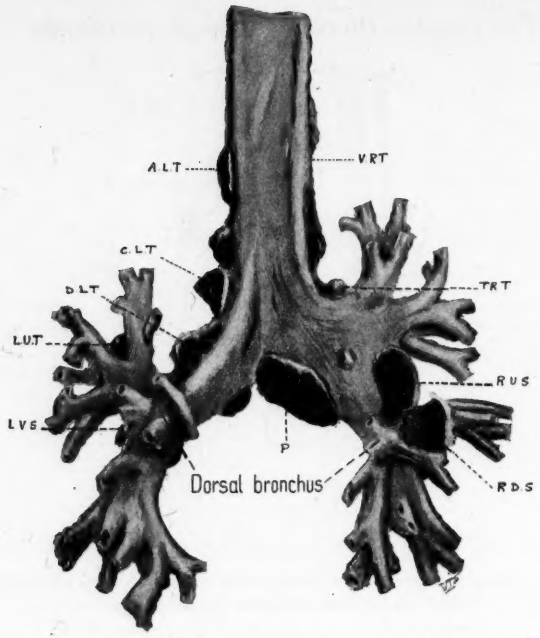

Fig. 9. Posterior view of the specimen shown in Fig. 8.

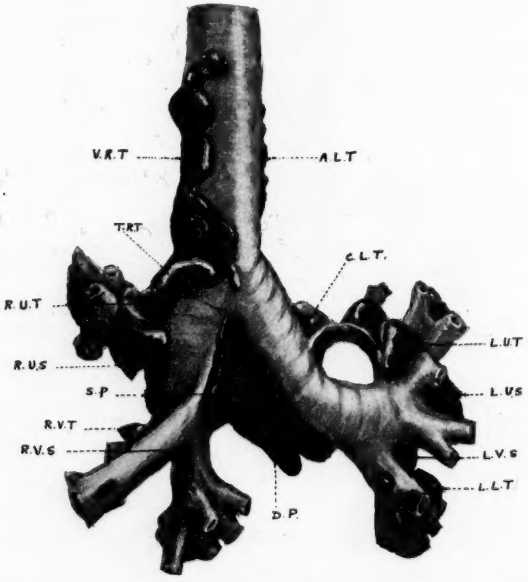

Fig. 10. A dissected specimen showing a bronchial tree of somewhat atypical form. Note the arcade of glands formed by the central portion of the left tracheal (*C.L.T.*) and the left upper inter-tertiary. The left upper inter-secondary is also shown in this specimen (*L.U.S.*).

downwards and backwards on the side of the trachea, and may lie either along the posterior margin of the group or cross deep to its upper part. The cardiac branches leave the vagus in the root of the neck and run downwards along the medial border of the glands in close contact with the anterior surface of the trachea.

2. *The left tracheal group* (*L.T.*) has no such definite arrangement as the right. There is a *central portion* which appears constant in all cases, lying under the arch of the aorta against the left side of the trachea, and in about half the cases there is an *ascending*, *descending* and *anterior* extension from this central part.

The central part (*C.L.T.*) usually consists of one large gland with its long axis from before backwards. It lies against the left lateral side of the trachea at the

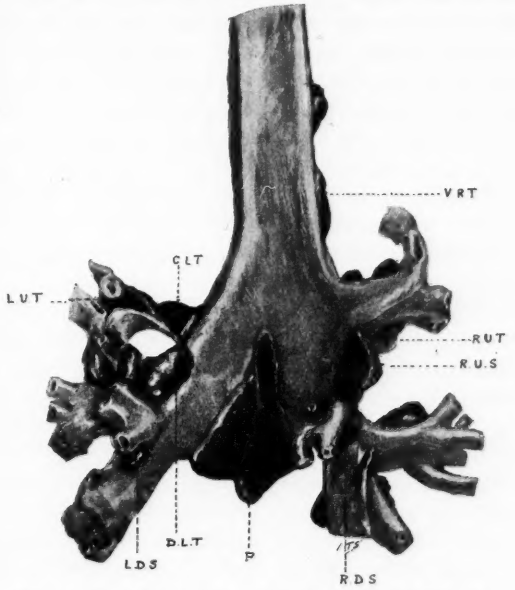

Fig. 11. Posterior view of the specimen shown in Fig. 10.

level of the carina, and as the left pulmonary artery runs outwards and backwards it grooves its under surface, while the aortic arch flattens it from above. In those cases where the glands are large, the left tracheal group may extend forwards across the front of the left pulmonary artery to meet the left upper inter-tertiary, and so, as on the right side, the pulmonary artery passes under an arcade of glands. The remains of the ductus anteriosus that runs from the bifurcation of the pulmonary artery to the descending arch of the aorta runs through the central portion of the left tracheal glands. The *ascending part* (*A.L.T.*) is a chain of two or three small flattened glands that spread upwards along the side of the trachea. They occupy a space that is triangular in cross-section with the trachea medially, arch of the aorta antero-laterally and the

oesophagus as it projects to the left of the trachea posteriorly. The recurrent laryngeal nerve runs upwards deep to these glands. The *descending part* (*D.L.T.*) is a chain of three to four glands that extends from the central part downwards and backwards along the superior border of the left bronchus to reach the dorsal inter-secondary gland below. They are large flat glands that stand out from the bronchus as a hedge along the posterior surface of the left pulmonary artery; they are flat behind but concave in front where they are in contact with the artery. Posteriorly is the pleura over the root of the lung, and behind that the descending aorta. The *anterior part* (*An.L.T.*) is very variable and often cannot be identified. There would appear to be three different conditions found: (*a*) one or two rounded glands lying behind the ascending aorta to the left of and independent from the right tracheal group; (*b*) a flat gland, closely adherent to the anterior surface of the trachea, that extends downwards and forwards to meet the superficial part of the inter-primary; (*c*) a chain of flat glands that runs down the anterior surface of the left bronchus for a short distance and lies underneath the left pulmonary artery as it crosses the bronchus.

The size and relation of the two tracheal groups is somewhat modified by the relation of the arch of the aorta and pulmonary artery to the level of bifurcation of the trachea. Usually the under surface of the arch of the aorta is at the same level as the internal bifurcation (carina), but it may vary as much as one centimetre above and below. As the result of a high arch (1) the vertical part of the right tracheal runs further upwards on the anterior surface of the trachea before winding laterally, and (2) the gap between the under surface of the arch and the upper surface of the left pulmonary artery is increased with consequent increase in the vertical height of the central part of the left tracheal. In one case the arch bore its usual relation to the carina, but the pulmonary artery was abnormally low and in consequence the right tracheal glands extended down in front of the inter-primary and the left tracheal were enlarged. With a low arch and normal pulmonary artery, the central part of the left tracheal is squashed between the arch and the trachea.

In the mediastinum a variable amount of fibro-fatty tissue is found in association with the glands, but in the hila practically no fat is found, and lung tissue touches the surface of the glands that are not in contact with bronchi or vessels.

The inter-primary group (*P.*) is wedged between the left primary bronchus and the right primary and main secondary bronchus. It consists of a deep and superficial layer of glands.

The deep part (*D.P.*) is the largest, being about seven-eighths of the whole group and consists of one large mass of glands that is wedged into the angle and extends down to the level of the ventral bronchus on the right but only half way down the left primary bronchus; below this on the left side are one or two discrete glands that extend down to the level of the upper bronchus.

The superficial part (*S.P.*) consists of three or four small flat glands that lie on the right side of the deep part and partly overlap the anterior surface of the right primary and main secondary bronchus.

The group as a whole is triangular in outline, the margins in contact with the bronchi are as thick as the bronchi but taper down to a thin free margin below. The posterior surface is flat and on the same plane as the membranous part of the trachea and bronchi. Anterior to the group is the pulmonary artery above and the oblique sinus and the upper part of the left auricle below. Posteriorly is the oesophagus.

The right upper inter-secondary group (***R.U.S.***) appears to be constant in all specimens. It consists of one or two glands that are wedged into the angle between the right upper and main secondary bronchus, and they extend down the lateral border of the main secondary towards the dorsal bronchus.

In cross-section they are triangular with the base of the triangle against the lateral side of the bronchus and the apex pointing outwards. The descending limb of the right pulmonary artery lies at first anterior and then antero-lateral to the glands; it separates them from the ventral lobe bronchus and grooves their anterior surface. The branch of the artery to the dorsal "lobe" cuts a deep notch in the glands as it passes backwards. The lung tissue and the oblique fissure lie postero-lateral to the glands.

Above, this group may be in contact with the upper inter-tertiary and below with the dorsal inter-secondary gland or separated from it by the artery to the dorsal "lobe."

The right ventral inter-secondary group (***R.V.S.***) was present in all but one case, and it usually consists of a solitary gland wedged into the acute angle formed by the ventral and lower bronchus. A prolongation of the gland winds round the lateral surface of the ventral bronchus and lies on the anterior surface of the descending pulmonary artery; from this lateral prolongation a chain of lymphatics runs onto the superior surface, where it is joined by a similar pigmented chain of lymphatics coming from the ventral inter-tertiary and together they run on into the inter-primary. Posterior to the gland is the pulmonary artery and below, medially and laterally, it is in contact with lung tissue.

The right dorsal inter-secondary group (***R.D.S.***) is a small solitary gland lying on the lateral surface of the dorsal bronchus at its origin. It may extend downwards for a few millimetres along the lateral border of the lower bronchus and upwards to meet the upper inter-secondary chain. It is flattened between the artery in front and the dorsal bronchus and lung tissue behind.

The left upper inter-secondary group (***L.U.S.***) is in the angle between the upper and ventral secondary bronchi of the left upper lobe. But as the upper bronchus is very short and the upper inter-tertiary glands are situated on its superior surface, they are usually indistinguishable from the inter-tertiary group and will be considered with them.

The left ventral inter-secondary group (***L.V.S.***) is, like that on the right, found constantly in all cases, and it consists of a large gland situated in the angle between the left ventral and lower bronchi. It sends a flat process backwards underneath the descending part of the pulmonary artery towards the dorsal

inter-secondary gland, and another process may be found winding around the back of the ventral bronchus to meet the upper inter-tertiary glands. Behind the gland runs the pulmonary artery, and the branch to the ventral "lobe" may notch the gland; anterior to it the lower part of the upper pulmonary vein crosses from the ventral "lobe."

The left dorsal inter-secondary group (***L.D.S.***), is, like that on the right, a small gland situated on the lateral side of the dorsal bronchus at its origin and is often continuous above with the descending part of the left tracheal. Antero-laterally is the artery and posteriorly the dorsal bronchus and lung.

The right upper inter-tertiary group (***R.U.T.***) is a mass of gland tissue situated on the anterior surface of the right upper bronchus and insinuates itself between the three principal tertiary branches. Medially it is wedged into the angle between the ascending and descending branches of the right pulmonary artery. The ascending branch separates it from the transverse part of the right tracheal group, but in some cases the glands spread across the front of the artery to meet a similar process coming from the right tracheal group. Below it is frequently found in contact with the upper inter-secondary gland. Several deeply pigmented lymphatics can be seen running from this group to the inter-primary. Anteriorly is the upper right pulmonary vein and the lung tissue.

The left upper inter-tertiary group (***L.U.T.***) is a mass of gland tissue situated on the superior surface of the left upper bronchus between its tertiary divisions, and, in conjunction with the upper inter-secondary, it may extend like a collar around the upper bronchus. In cases with large glands this group may extend across the left pulmonary artery to communicate with the left tracheal. In one case a thin chain of glands was found running upwards and inwards from the inter-tertiary to join the inter-primary group. The upper pulmonary vein and lung tissue cover the anterior surface of the glands.

The inter-tertiary glands of the lower and ventral lobes are small and variable; they do not adhere strictly to angles between the dividing bronchi but may be found adherent to the sides of the bronchi. But the ventral and lower inter-tertiary, inter-quaternary and other intra-pulmonary collections of lymphoid tissue have not been considered in this work.

I agree with Poirier, Cuneo and Delamere in their description of the other visceral glands in the thorax, namely, (1) *the anterior mediastinal group* (*A.M.*) that constitute a mass of glands situated on the anterior and lateral surface of the arch of the aorta and detach themselves in chains that run upwards toward the base of the neck. The left vagus nerve runs along the posterior margin of this pre-aortic group and the left phrenic nerve crosses it superficially. Both phrenic nerves are in contact with gland tissue, the left to the pre-aortic and the right to a solitary gland found lying between it and the pulmonary vein in front of the root of the right lung which gland might be called the "*anterior radicular*" gland (A.R.). In several pathological cases the nerve was found firmly adherent to the glands; this fact accounts for the difficulty sometimes encountered in the operation of evulsion of this nerve, and I have also seen it responsible

for atrophy of the hemi-diaphragm. (2) *The posterior mediastinal glands* consist of two or three long, oval glands that usually lie on the anterior surface of the oesophagus between it and the trachea or pericardium.

SUMMARY

1. A terminology for the bronchi is presented that is based on their area of distribution rather than on the morphology of the lung. Four secondary bronchi are described on each side, i.e. upper, ventral, dorsal and lower.

2. A new and simple terminology for the bronchial glands is presented.

3. The glands found in the mediastinum and hila of the lungs are described in detail.

4. Brief reference is made to the other visceral glands in the thorax.

REFERENCES

(1) Aeby, Ch. (1880). *Der Bronchialbaum des Menschen und der Säugetiere.* Leipzig.
(2) Dickey, J. Stuart (1911). *Applied Anatomy of the Lungs and Pleural Membranes.* London.
(3) Ewart, William (1889). *The Bronchi and Pulmonary Blood Vessels.* Baillière, Tindall and Cox, London.
(4) Narath, A. (1901). "Der Bronchialbaum der Säugetiere und der Menschen." *Bibliotheca med.* A, Heft 3, Stuttgart.
(5) Nelson, H. P. (1930). "Irradiation of the tracheo-bronchial lymphatic glands." *The Lancet,* Nov. 22, p. 1118.
(6) Poirier, Cuneo and Delamere. (1904). *The Lymphatics.* W. T. Keener and Co., Chicago.
(7) Schwalbe, Ernest (1912). *Die Morphologie der Missbildungen.* Jena. Teil III. Pp. 829-836.

EXPLANATION OF FIGURES

R.T Right tracheal.
T.R.T. Transverse right tracheal.
V.R.T. Vertical right tracheal.
L.T. Left tracheal.
A.L.T. Ascending left tracheal.
C.L.T. Central left tracheal.
D.L.T. Descending left tracheal.
An.L.T. Anterior left tracheal.
P. Inter-primary.
S.P. Superficial inter-primary.
D.P. Deep inter-primary.
R.U.S. Right upper inter-secondary.
L.U.S. Left upper inter-secondary.
R.V.S. Right ventral inter-secondary
L.V.S. Left ventral inter-secondary.
R.D.S. Right dorsal inter-secondary.
L.D.S. Left dorsal inter-secondary.
R.U.T. Right upper inter tertiary.
L.U.T. Left upper inter-tertiary.
V.T. Ventral inter-tertiary.
D.T. Dorsal inter-tertiary.
L.T. Lower inter-tertiary.
Q. Inter-quaternary, etc.
A. Aorta.
A.M. Anterior mediastinal glands.
A.R. Anterior radicular gland.
Az.v. Vena azygor major.
L.A. Left auricle.
L.A.P. Left auricle appendage.
L.B. Left bronchus.
L.D.B. Left dorsal bronchus.
L.C.C. Left common carotid artery.
L.P.A. Left pulmonary artery.
L.V.A. Artery to left "ventral lobe."
O.S. Oblique sinus.
P.A. Pulmonary artery.
R.B. Right bronchus.
R.D.B. Right dorsal bronchus.
R.P.A. Right pulmonary artery.
R.V.A. Artery to the right ventral lobe.
S.V.C. Superior vena cava.
Tr. Trachea.
T.S. Transverse sinus.

THE POTENCY OF THE PHARYNGEAL ENTODERM

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THE pharyngeal entoderm has long been famous as the seat of formation of the branchial arches and their derivatives. These structures remind us of our origin from water-breathing forms, and are always quoted as one of the best anatomical proofs of evolution, and as a striking proof of the biogenetic law that each organism repeats, albeit in a compressed and abbreviated manner, the history of its origin. This region is of course also the seat of some of the more spectacular ductless glands.

EARLY MORPHOGENESIS OF THE PHARYNGEAL ENTODERM

A scientific morphology can be based on only two lines of enquiry, viz. an investigation of the history of the structure and a knowledge of the functions it performs.

Descriptive and experimental embryology has established the fact that the body of the vertebrate embryo is laid down in a definite order due to the successive activity of three great centres of growth. In the course of its development the embryo reaches a stage when it becomes a bilaminar slightly elongated body enclosing a central cavity, the archenteron. The place where the two laminae are conjoined is known as the blastopore. The margins of this blastopore, owing to the proliferation of the cells which compose it, adds further to the length and volume of this original cavity. The portion so added is called the deutenteron. In the formation of the axial organs of the embryo these two parts behave very differently.

The archenteric region builds up the apical extremity of the body; actually the parts in front of the notochord, a line which in the human body passes obliquely through the posterior wall of the pituitary fossa. This region includes the fore-brain vesicle, i.e. the cerebrum and diencephalon or thalamic region, sense organs like the optic and olfactory, the front wall of the pharynx and the buccal cavity.

The cells of the blastopore itself multiply and add to its volume by interstitial growth. This interstitial growth constitutes the second region of growth, and it produces the *head region* of comparative anatomy. The head in comparative anatomy means the zone which is bounded in front by the origin of the trigeminal nerve and behind by the caudal limit of the vagus. This cephalic region includes pre-eminently the branchial region.

Around the lateral part and caudal to the remains of the blastopore there is a zone of appositional growth, an area in which increase in size is due to the

addition of cells from the neighbouring parts. This zone of appositional growth forms the third growth centre, and from it the trunk and tail are formed. It is the seat of the alimentary, the locomotive, and the genito-urinary apparatus. The nervous system is simple without important differentiations. The peripheral nerves merely repeat themselves, and there are no organs of special sense and no branchial windows.

These three great stages succeed each other, though indeed there is some overriding, and it is certain that they occur in all vertebrate embryos. The head is not a transformed trunk but a primary ontogenetic product, while the trunk and tail are secondary diversities.

IMPLICATIONS OF THIS MODE OF DEVELOPMENT

These facts of development must be explained by the origin and evolution of the vertebrates, though all inferences must be checked by observation and experiment wherever possible.

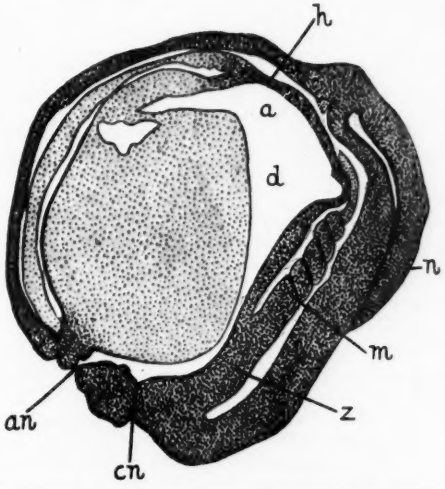

Fig. 1. Sagittal section through a frog embryo showing the zones of differentiation. *a.* archenteron; *an.* anus; *cn.* neurenteric canal; *d.* deutenteron; *h.* hypoblast; *m.* mesoblast; *n.* central nervous system; *z.* zone of apposition. (Brachet, *Traité d'Embryologie des Vertébrés*, 1921.)

This general account of morphogenesis is implicit in the work of Gaskell on the origin of the vertebrates. He recognised the fore-brain as the oldest region of the vertebrate body. Gaskell has been criticised for the excessive ingenuity with which he derived the vertebrate from an already highly specialised form represented more or less by the still existing king crab. At the moment there is an increasing reluctance among some anatomists to admit the derivation of higher forms from types obviously akin but already specialised in some way. The idea of succession is dropping out of evolution, and with the departure of

the notion of sequence the term evolution becomes almost meaningless. Some would deny the closeness of the chimpanzee-gorilla stock to Man and seek for his origin in more remote and more generalised types. The same way of thinking affects conceptions of the origin of the vertebrates. Nevertheless the insight embryology has given us of the order of development shows that Gaskell cannot have been very far from the truth, and we need not be so frightened of the idea of sequence in evolution. As for generalised types, it is to be asserted that some degree of specialisation is a condition of existence at all.

The precedence of the brain in evolution of vertebrates has already infected neurology and our way of thinking about behaviour. A theory of behaviour founded on the spinal reflex must be an abstraction, for the brain is not a modified spinal cord. General impressions and total responses precede individual and local responses.

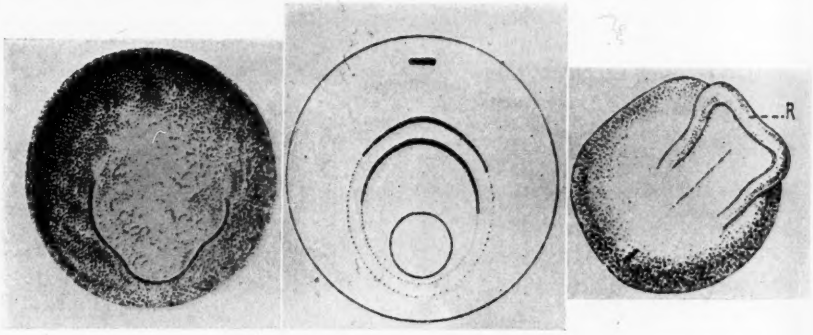

Fig. 2.

A. Frog gastrula looked at from the lower pole. In this non-pigmented area, the grey crescent, the blastopore has formed and is shaped like a horseshoe. (Brachet.)
B. This is a schematic representation of the manner in which the blastopore closes. (Morgan.)
C. The short black mark indicates the position of blastopore when almost closed. The elevated margin labelled *R* is the transverse medullary fold, and the area between this cerebral fold and the blastopore will become the medullary tube. (After Hertwig.)

The order of events outlined above also affects the vegetative nervous system. As a matter of expectation from the above and also as a matter of observation the system is comparatively more recent, and the order of origin and development is vagal, sacral and lastly thoracolumbar.

DIFFERENTIATION AS AN INDUCED PROCESS

Thus far we have been led to conclude that the front end of the pharynx belongs to the oldest and first-formed part of the vertebrate body, while the branchial region occurs in the deutenteric region, the second portion to be laid down by interstitial growth in the vicinity of the blastopore.

Recent investigations in the field of experimental embryology have revealed how development in some part at any rate depends on the action of certain inducing structures. We know from the work of Brachet that the formative materials out of which the later organs will be formed reside in the cytoplasm of the ovum and become definitely localised when the ovum is penetrated by the sperm.

With the entrance of the sperm several major events are decided. Firstly, there is the contribution of paternal chromosomes; secondly, the stimulus to cell division; thirdly, the inception of a definite localisation of the developmental factors; and, fourthly, a coincidence between the point of entry and the plane of the first division of the ovum which corresponds to the axial symmetry of the right and left halves of the body.

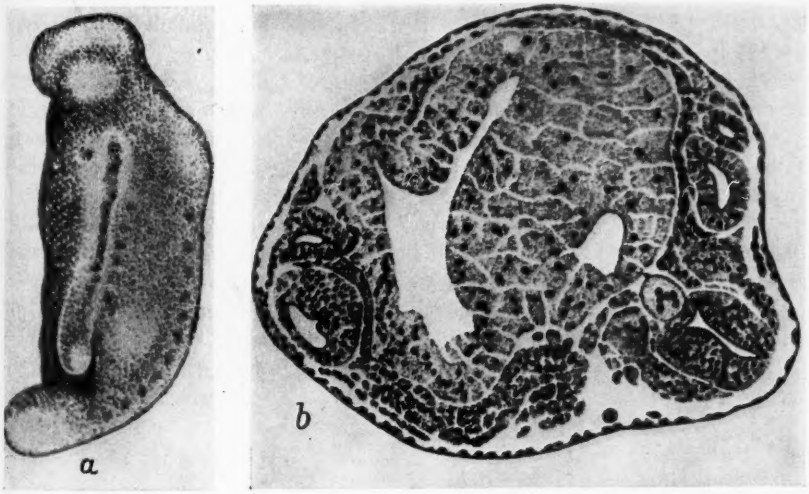

Fig. 3. The "organiser" from the dorsal lip of the blastopore has been implanted from one species of amphibian into another. Fig. *a* on the left shows a secondary embryo developing on the left side of the host. The secondary embryo consists of medullary tube, somites, otocyst and tail. *b*. The same embryo seen in section. On the left the axial organs of the host are seen; on the right the medullary tube and otocyst of the secondary embryo. (Spemann.)

In the fertilised frog's egg there develops an area free from pigment known as the grey crescent. This by observation and experiment is known to outline the future blastopore of the embryo. The cranial lip of the blastopore occupies the greater part of the crescent, while its lateral lips follow the horns of the crescent.

The margins of the blastopore approach each other by an eccentric growth, and all the dorsal organs of the embryo come to occupy the area in front of the blastopore constituting what is known as the dorsal plate. It is here that the medullary tube, the notochord, and the somites which become the myotomes will be differentiated. Just in front of this area the transverse cerebral fold out

of which will be formed the prechordal head region, the first growth area, appears.

These localisations are now known for many organisms, and may, with some minor variations, be regarded as occurring in all amphibians. With a knowledge of the localisation of developmental factors the way is open for further investigation. So far it seems certain that the development factors reside in the ovum and have nothing to do with the sperm. The order of localisation and the time sequence are still obscure and nothing is known of their physicochemical nature.

The dorsal organs, medullary plate, notochord, somites, etc., do not undergo spontaneous development. They are induced by what Spemann has called the "organiser." Spemann used this term organiser to indicate that part of the cranial lip of the blastopore which acts as a morphogenetic centre inducing the development of these dorsal organs. Spemann's experiments are many, but amongst the most decisive are those in which it was shown that material, which, if left in its own position in the embryo, would have become, say, ventral abdominal wall, is changed when brought into proximity with the organiser into nervous system, notochord, muscle, etc. Or, conversely, when the organiser is implanted into some other region of the embryo where these dorsal structures are not found, their formation is immediately excited. Most striking of all was the induction of these dorsal organs by the organiser when it was transplanted into another embryo of a different species. The embryos chosen were differently pigmented, so that the events could be easily followed. A double embryo was formed with two sets of dorsal organs. That the organiser actually changed cells of the host into these structures could be seen easily, for the difference in pigment made identification of host and transplant evident.

The essential elements in the organiser reside in the entodermal cells. The overlying ectoderm has no effect. This cannot be without significance, for the endocrines of the pharynx are mainly entodermal in origin. The mode of action of the organiser is still under investigation, but it is to be noted that it is not preformed in the ovum but is formed during development, and secondly that its action is only effective at a certain time in development.

It is the body of the embryo that comes into being in this manner, the branchial region and the trunk, while the first area, the prechordal region, seems to arise in an altogether autonomous manner.

One other observation may be mentioned for its future clinical interest. Brachet found that if the lateral part of the grey crescent in the frog's egg (this corresponds to the region of Spemann's organiser) be injured by a heated needle, then cell proliferation might follow but no differentiation. Such an embryo lives for only a limited period. Investigations in this manner have shown that the size of the organs which will be formed from a particular region depends on the amount of organising substance they have received, and that organs which have been reduced in size in this manner never regain later what they earlier lost.

At the moment we cannot piece the many facts gathered from experimental embryology into an ordered account of development. The genes of the chromo-

somes must be in some way parallel to the organisers. Then other organs, so far as we know, proceed with their development without induction, such as the prechordal head. For all the stages later than gastrulation we know of no other morphogenetic organs until we come to the ductless glands. There is perhaps one sort of exception to this. Some organs which themselves may have been induced may acquire the property of inducing secondarily others. The relation of the optic cup to the overlying ectoderm seems a case in point. For experiment shows that the cup has the power in some cases of inducing the overlying ectoderm, normal or transplanted, to form a lens.

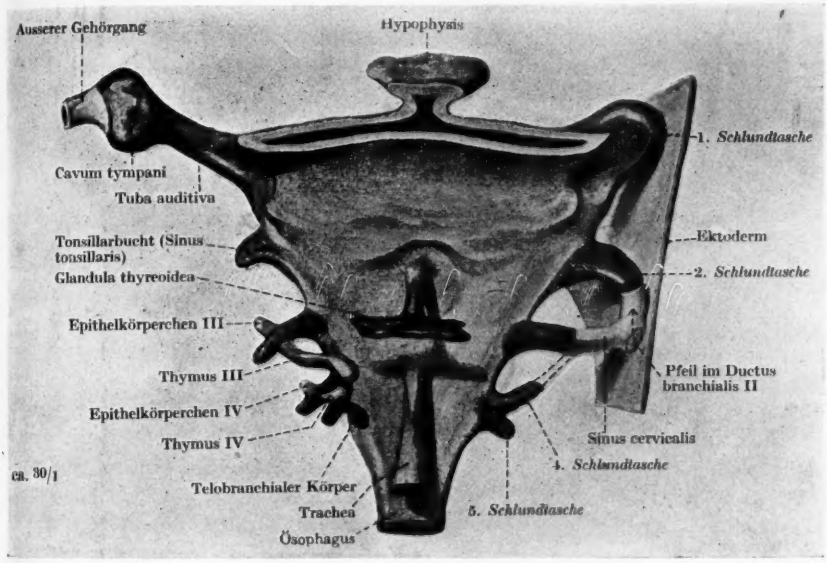

Fig. 4. A representation of the pharyngeal derivatives.

THE DEVELOPMENT OF THE BRANCHIAL REGION

Perhaps we may regard the pharyngeal entoderm as a secondary organising region. It will be necessary first to discuss its development before we can examine this idea further.

Though the number is greater in the more primitive vertebrates only four branchial arches, five depressions and six aortic arches have been recognised in Man. The branchial depressions immediately succeed the corresponding arches, the fifth depression being rudimentary. In those animals with branchial respiration the depressions become clefts, but in all Mammals the entoderm remains in apposition with the ectoderm, a cleft only appearing occasionally in the second.

The external form of the neck is affected mainly by the first and second arches, for these rapidly increase in size and so expanding come to overlie the remaining arches and thus bound a depression known as the cervical sinus. Within this sinus there will be the external depressions from the second to the fifth, while its prominent upper margin will be formed by the second arch. The caudal boundary is formed by the thorax towards which the second arch has grown.

Internally the entodermal depressions increase in what corresponds to the lateral wall of the pharynx. Here the depressions become drawn out into narrow ducts whose blind ends are in apposition with the ectoderm, and in the case of the second to the fifth the place of apposition is in the upper part and floor of

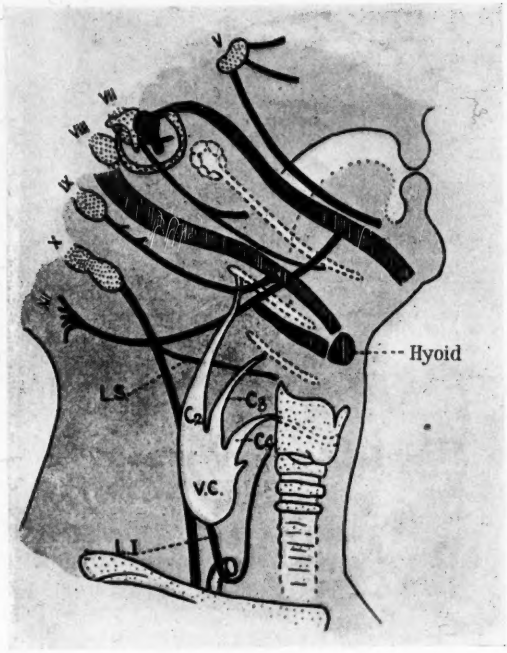

Fig. 5. A schematic representation of the gill arches (vertical hatching), the branchial depressions (clear slits with dotted margins), the relations of the nerves (solid black). *V.C.* is the cervical sinus, and the scheme shows its connections with the entodermal depressions. (After Moatti.)

the cervical sinus. These ducts soon lose their connection with the pharynx, this process starting caudally and then later involving the third and second pharyngeal ducts. With the loss of the pharyngeal connections buds of entoderm are left enmeshed in the mesenchyme of the neck. At the same time the second arch fuses with the thorax and the ectoderm lining the cervical sinus disappears. These changes are completed by the end of the first month of intrauterine life.

From these discrete entodermal remnants organs of great interest are formed. The tonsil arises from the extremity of the second duct. The blind end of the third forms a dorsal and a ventral part. The dorsal part becomes one pair of parathyroids while the ventral becomes thymus. The fourth behaves in a similar manner, but in Man the ventral contribution to the thymus seems to have dropped out.

The apex of the first depression is found in the middle ear at the tympanic membrane. The last forms the ultimo-branchial body and can be traced for some time in the lateral lobe of the thyroid gland, from which it can always be distinguished. It disappears finally without forming any part of the thyroid.

The Eustachian tube is supposed to represent the first cleft, the supratonsillar fossa a remnant of the second, the piriform sinus the third above the superior laryngeal, and the fourth below this nerve.

The thyroid arises from the median entoderm of the pharyngeal floor anteriorly just behind the stomodeal membrane on a level with the first arch, and grows ventrally and caudally. In its growth it undergoes some caudal displacement, and from being ventral and in front of the hyoid arch it comes to lie dorsal to this, thus bringing the thyroglossal tract dorsal to the hyoid bone. From the blind extremity of the tract epithelial cords are first produced. Then these become broken up and colloid appears in the columns which become transformed into vesicles.

THE NEURAL CREST AND THE BRANCHIAL REGION

This presentation of the development of the branchial region has followed orthodox lines, but there are a number of facts and observations to be mentioned which should convince us that the whole of the truth is not to be compressed into any merely schematic arrangement. Useful such may be for making a complicated process intelligible, useful in giving a general idea of the anatomy of the anomalies of this region, but it leaves out the more dynamic aspects of pharyngeal growth and development.

There are a large number of observations on lower vertebrates, on the monotremes, and on Mammals and Man which have drawn attention to the relatively enormous mass of neural crest tissue which is proliferated in the region of the hind-brain, to the extensions and migrations which this undergoes, and its apparent metaplasia into a form resembling mesoderm. For this reason it is termed mesectoderm. The suspicion that this tissue of ectodermal origin took some part in the formation of the branchial region was put to experimental test. Excisions of this material in the Amphibia and a study of the resulting defects in growth have shown quite clearly that the branchial skeleton is derived from material which belong to the neural crest.

This applies to the lateral portions of the branchial skeleton. The median part of the neck has a different origin. This part of the neck is derived from tissue belonging to the pericardial region. This has recently been confirmed by Fraser in the study of the human embryo, for he has traced the origin of the

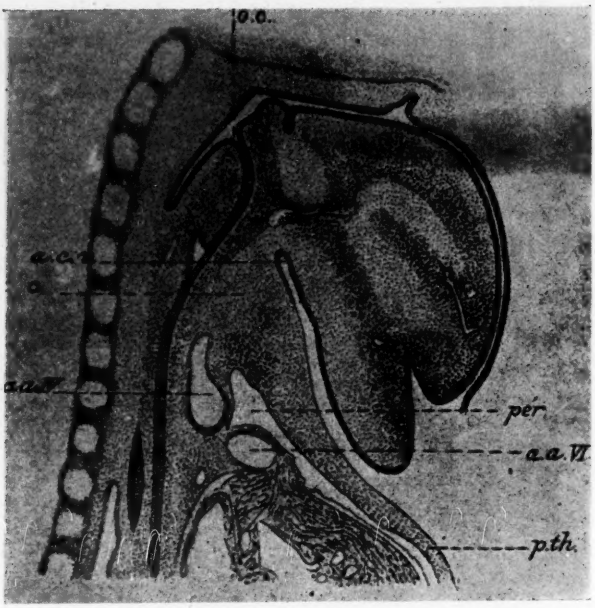

Fig. 6. A sagittal section of a rabbit embryo illustrating how the median tissues of the neck are formed from a prepericardial (*p.th.*) lamina that extends up to the cervico-facial angle (*a.c.v.*). (After Vialleton.)

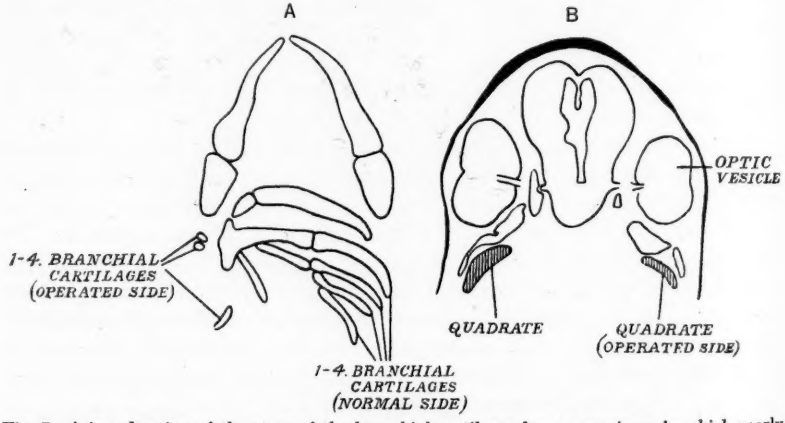

Fig. 7. A is a drawing of the state of the branchial cartilages from a specimen in which nearly the whole of the neural crest had been removed eighteen days previously. Only three small cartilages appear on the operated side. B is a section of a similar specimen at the level of the optic vesicles showing the relative sizes of the quadrate when the neural crest is removed on one side. (L. S. Stone.)

middle line of the neck from the epicardial tissues. As a matter of fact, only the first and second arches approach the middle line of the neck, the third and fourth are displaced laterally: i.e. the part of the neck most affected by the branchial arches is in the submandibular region. It is possible that this metaplasia and differentiation of ectodermal tissue into a cartilaginous skeleton is due to induction by pharyngeal entoderm.

FURTHER REMARKS ON THE BRANCHIAL DEVELOPMENT

The branchial region is a very fleeting affair. While the more caudal arches are being formed the more cranial ones are disappearing. Their very existence is evanescent, for the invasion of mesodermic tissues soon makes them unrecognisable. The coincidence between the internal and external depressions is soon lost, and the arches as such have disappeared before the cartilaginous skeleton has appeared. The aortic arches are never definite vessels in the human embryo. They form only a capillary net in which the blood flow through them is made difficult by other factors. Again these vascular arches are never present simultaneously, the cranial ones having gone before the caudal ones have appeared.

The temporary nature of these arches and depressions, the quick changes induced by the invasion of mesoderm make difficult the precise interpretation of the later events in the anatomy of the neck. Fraser has drawn attention to the inaccuracy of the orthodox description that limits the Eustachian tube to the first cleft. Further, he has shown that the cervical sinus is a much more restricted affair than is usually supposed. The pillars of the fauces and the supratonsillar fossa usually regarded as parts of the second arch, the second entodermal depression, and the third arch are regarded by some as altogether secondary and not truly related to the branchial system.

Just as the morphological results of branchial development fit uneasily into our teaching schemes, so also is its potency for developing organs not so focal as these schemes suggest. Not only is there aberrant tissue associated with the usual organs derived from the entoderm, as indeed there is with all organ development of the body, but an appeal to comparative anatomy shows a wide variation in the origin of the various derivatives. The pituitary is generally regarded as being of ectodermal origin starting immediately in front of the dorsal attachment of the membrane that separates the mouth from the pharynx, but in marsupials and other species Watson found that it was, in part at any rate, of entodermal origin, and this variation is common in lower vertebrates.

In some of the lowest vertebrates (*Petromyzon*) every branchial depression forms a thymus, and throughout the vertebrates there is the greatest variation in its mode of origin. In Reptiles and Birds, for instance, it comes from the dorsal element of the thymopharyngeal duct, while in Mammals it comes from the ventral.

These variations are to be interpreted as evidence of the general potency of the pharyngeal entoderm.

HETEROTOPIA

The complicated method of development which occurs in the neck affords a strong presumption that inclusions of entoderm and less commonly of ectoderm in unusual positions would be a common matter. In addition to the processes already alluded to, the migration of premuscle tissue and the constricting effect of the loop around the arches formed by the hypoglossal nerve afford additional causes for displacement.

The estimation of the frequency of displaced elements entails a laborious investigation, and the few attempts which have been made give discordant results. Some have claimed that heterotopia is no more frequent in the neck than elsewhere, while another has recorded the frequency of such inclusions as twenty-six in fifty-five investigations.

In addition to such inclusions and displacements of embryonic material, there exists also in the neck vestigial organs like the ultimobranchial bodies. The proper fate of this body is to disappear about the seventh month of intra-uterine life, but observations have been published in which it is stated that this organ persists and forms cysts containing a colloid-like substance and also assembling tissue about it with a structure like that of the thymus.

Clinical experience encounters not only fistulae but also cysts which may become papilliferous and invasive and in which colloid may be found and also lymph nodes and Hassall's corpuscles, and even cartilage may occur.

Histological investigation has disclosed the fact that displacement, persistence, and metaplasia are not infrequent associations of the developmental process in all parts of the body. There seems to be some evidence that these events are common enough in the neck, but no reason for believing that they are more likely to persist or become invasive here than elsewhere in the body. This seems to suggest that the control of the behaviour of these heterotopic tissues resides elsewhere than in the displaced tissues. The frequent presence of lymph nodes and Hassall's corpuscles has suggested to some that it is always thymic tissue that is displaced. This kind of metaplasia is so frequent wherever entoderm interacts with mesenchyme that it should be regarded as a general phenomenon and not a specific attribution of the thymus.

It seems at the moment that any portion of the pharyngeal entoderm may be the source of an aberrant entodermal inclusion, and any such inclusion, if it grows, is likely to assume a cystic papilliferous form and excite a lympho-epithelial reaction. This again we would attribute to the special quality of the entoderm of the pharynx.

POSSIBLE FUNCTIONS OF THE PHARYNGEAL ENTODERM DURING EARLY EMBRYONIC LIFE

In addition to furnishing several well-known ductless glands, the suggestion has been hinted occasionally that perhaps the pharyngeal entoderm has some inducing action during the early development of the embryo. No proof can be advanced at the present moment, but perhaps the idea is worth investigation.

At any rate it is unwise to regard the transformations of the branchial region as being merely of historical interest. A branchial region occurs in all vertebrates, but only in the Icthyopsida (the Fishes, and Amphibia) do they function as respiratory organs. Some have gone so far as to deny their evolutionary significance if they function only in such a small fraction of the vertebrate kingdom. Kranichfeld has suggested that some embryonic functions reside in the pharyngeal region. He brings some general arguments, but specifically suggests that the so-called epibranchial placodes may be such embryonic organs. These are cell accumulations occurring along the dorsal extremities of the arches in association with the seventh, ninth and tenth nerves. It is generally supposed they form the lower of the two ganglia to be found on these two nerves, but their precise origin and function have not yet been determined.

Pende has described a series of epithelial structures found in the neck tissues of later embryos and distinct from the known glands of the neck. His histological studies of these organs led him to suggest that they might function as embryonic organs. His anatomical observations have not, however, been confirmed by anybody. It is, however, true that one does encounter organs of an epithelial nature in the neck in later embryos which are distinct from the known glands, which have all the appearances of active structures and not those of degenerating ones.

On general grounds it might be supposed that the higher vertebrates would base their more complicated development on a larger series of inducing organs than the simpler vertebrates, and the pharyngeal entoderm is the most likely place to look for them.

THE FUNCTION OF THE PHARYNGEAL ENTODERM IN LATER EMBRYONIC LIFE

Dependence of foetus on its own ductless glands

The anatomist naturally wishes to formulate some general notion of the significance of the endocrine organs derived from the pharyngeal entoderm.

The activities of the endocrine glands have been classified in various ways, but broadly they can be considered as either morphogenetic or physiological. At the very outset one is confronted with the difficulty that it is not always possible to distinguish structurally those elements which form morphogenetic secretions and those which have regulatory functions, and indeed it is possible that one and the same substance may do both.

In trying to get some idea of the rôle of the morphogenetic secretions in determining the growth and form we can appeal to the order and mode of the development of the vertebrate body as outlined above. Further, we can apply Child's law of gradients, for development does pursue a cranio-caudal progression and there is some evidence that the physiological gradient slopes in the same way.

The pituitary belongs to the prechordal region which we have seen is the

oldest part of the vertebrate body and the first to be developed, and since this has an inductive effect on the later growth centres and indeed gives rise to an organiser that induces the formation of notochord, medullary plate, somites, pronephros, etc., we might expect to find that the pituitary plays a dominant rôle both in regard to its morphogenetic action and its control of the later ductless glands. Therefore the order of appearance of these glands and the inception of their activity become of some interest.

It might seem at first sight that amongst placentals the embryo would have no need to invoke its own ductless glands, for the maternal circulation would supply these secretions if necessary to development, and indeed there is some clinical evidence that insulin passes from the foetus to a diabetic mother. Also the sporadic cretin or, perhaps better, infantile myxoedema, the athyreotic type, which appears not earlier than three months after birth, might be regarded as having subsisted on the secretion of the maternal thyroid before birth.

Nevertheless we have the broad fact that endocrine organs are peculiar to a fragment of the animal kingdom, namely the vertebrates. Their existence amongst the invertebrates has never been proved and all the evidence is to the contrary. In the group of vertebrates it is only amongst the Mammals the possibility of interchange of these secretions occurs. In the Fishes, the Amphibia, the Reptiles and the Birds, the development of the embryos must proceed in the absence of maternal hormones. Experimentally McCleod has found no evidence in dogs that insulin passes from the foetus to the mother. Furthermore, these secretions are non-specific, and the fact that a sample from any vertebrate will affect any other vertebrate suggests that their developmental rôle would be the same in them all. On general grounds an interchange seems improbable, for it would be necessary to imagine a mechanism that let through some and not others, that regulated the amount passed through, and became active at only the appropriate time. Despite the difference in the degree of response to thyroid treatment between the endemic and sporadic cretin, yet in both the thyroid defect is the most important fact and endemic cretinism arises *in utero*. Sporadic cretinism is better understood by regarding the term athyreotic as relative and not absolute, and the time of onset as being indicative of the degree of anaplasia. Finally we might urge that if the foetus receives its hormones from the mother there would be reason to suppose that they might not develop at all, for a resected thyroid does not hypertrophy if the hormone be administered. Thus we would conclude that the foetus does not receive its hormones from the mother.

ACTIVITY OF DUCTLESS GLANDS DURING DEVELOPMENT

It is not altogether certain that the endocrine organs assume their activity during foetal life. The pituitary comes into being as an embryological structure when the embryo is 2·5 mm. in length, while the evagination of the floor of the third ventricle appears at 10·5 mm. (the seventh week). According to Erdheim, eosinophile cells appear in the embryo when 29 cm. in length, and basophile cells

just before or after birth. Hammar has placed the appearance of eosinophile cells as early as the 20 to 25 mm. stage, but this needs confirmation.

The thyroid appears very early when the embryo is between 2 or 3 mm. in length. The degeneration of the thyroglossal duct, the formation of follicles and the appearance of colloid have all been followed. Colloid appears when the embryo has reached a length between 25 and 40 cm.

The histological evidence of activity has been supplemented by experimental enquiry. An extract of the glands has been obtained by rubbing up the glands from foetal pigs and testing this on tadpoles. The results obtained by this and similar methods have been somewhat discordant. Hogben and Crew's results agreed well enough with the histological evidence, and they concluded that these glands became active during the second half of pregnancy—at four months in the calf and at the third month in the sheep.

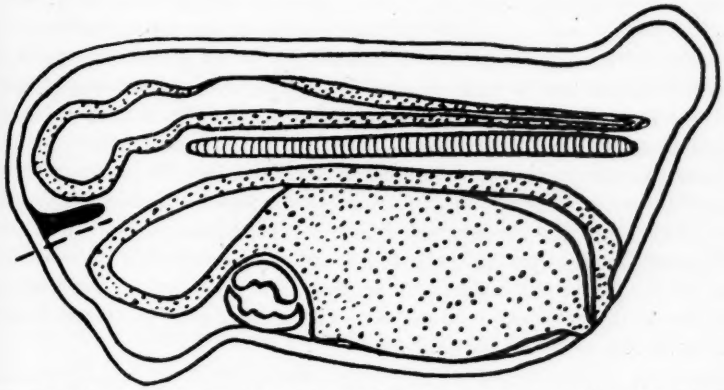

Fig. 8. A drawing of a median section of the amphibian larva to show the position of the pituitary anlage (black) and the site of the incision for its removal (dotted line).

DOMINANCE OF PITUITARY

The evidence obtained from experiments on the Amphibia is far more satisfactory and conclusive. Ablation experiments on amphibian larvae show definitely that even the earliest stages of development of the pituitary are essential for the development of the thyroid gland. The secretion of the anterior lobe of the pituitary must be present in the blood or development of thyroid does not take place. The buccal outgrowth of the pituitary includes the pars anterior, the pars intermedia and the pars tuberalis. The operation does not touch the pars nervosa. This does appear when the buccal anlage is removed, but its development is abnormal and retarded compared with the normal. Should, however, any part of the pars buccalis be left by the operation, then

the development of the pars nervosa becomes normal. Thus we conclude the development of the pars nervosa depends on the pars buccalis.

The effect on the thyroid of removal of the buccal outgrowth is to reduce its size to about one-sixth of the normal and it does not form or store colloid. Further, the metamorphosis does not occur in the absence of the buccal outgrowth. Such tadpoles can be got to metamorphose in two ways, one by feeding them with thyroid preparations or implanting the pars anterior. The pars intermedia has no effect and the pars tuberalis has not been tried. With the implantation of the pars anterior the power of the thyroid to store its colloid is regained. The hypophysis without the thyroid is powerless to induce metamorphosis. It is therefore concluded that, whatever be the rôle of the hypophysis, the secretion it forms works in co-operation with the thyroid and induces metamorphosis. The mode of co-operation is not a nervous one.

It is to be noted that amongst other effects induced by ablation of the buccal anlage is retardation of growth. Thyroid ablation does not stop the growth of tadpoles though metamorphosis does not occur. Evans got thyroidectomised rats to grow by giving them intraperitoneal injections of pars anterior, and concluded that cretinism in rats was due to a dysfunction of the hypophysis and thyroid. This point may be of clinical interest. Growth in tadpoles can be induced by the pars anterior in the absence of the thyroid.

From these amphibian studies it can be concluded that the pituitary and the thyroid are responsible for the continuance of development from a certain point onwards, this point being characteristic of each species.

These studies at any rate suggest that the dominant endocrine is the pituitary and not the thyroid as is generally asserted, and that the inference from its situation in the prechordal growth centre is not without significance in interpreting its rôle in development.

THE RELATIONSHIP BETWEEN THE MORPHOGENETIC AND THE PHYSIOLOGICAL SECRETION

The pars anterior with its eosinophile cells is the seat of the growth hormone of the pituitary, and at the same time it exerts an inducing effect on the pars nervosa. At the moment we need not discuss the problem as to whether it is the hypothalamus or the pars nervosa that exert the physiological effects of this region. Suffice it to say that comparative anatomy is in full accord with the present ideas centring round the hypothalamus. We can agree that the pars anterior is the growth-promoting element and the inducer of the thyroid activity, that the pars intermedia both on histological and experimental grounds is probably inactive. Nothing is known about the pars tuberalis at the moment.

The idea suggests itself that the vertebrate in adopting the hormonic method of growth first began with the morphogenetic elements and then met the physiological demands so engendered by the development of necessary catalytic and stimulating hormones. In some way like this the warm-blooded vertebrates

came into being. The twofold rôle seems clear enough in the case of the pituitary, but it is not easy to understand in the case of the thyroid.

It is true that the idea of a double secretion in the case of the thyroid has occurred to several. It is not difficult to imagine, though as yet impossible to prove, that the colloid is the morphogenetic secretion, while the follicular cells directly secrete into the blood stream the substance that elevates the metabolic rate. Histological evidence of this twofold activity has been presented by Bensley. There is some clinical evidence that exophthalmic and myxoedematous symptoms may occur in the same patients.

There is also the extremely interesting fact that thyroid preparations have no effect on the adult frog. There is some evidence of a rise in metabolic rate during metamorphosis, but after this event no effect can be discerned. We must suppose that the metabolic effect is acquired later in evolution presumably amongst the extinct Mammal-like Reptiles.

THE PARATHYROID

I would say nothing about the parathyroid, as its rôle seems to be primarily physiological—acting as a stimulant of the osteoclast. From the work of Dott and Fraser we can assign a place to these several glands in accordance with their relation to the cells of bone. They observed that the cartilage cells were unable to maintain themselves in the absence of the pituitary, while if the thyroid was removed the cartilage cells persisted but did not multiply. The parathyroid comes into bone formation at a later stage, that of modelling. Thus the cranio-caudal situation of these organs has a corresponding sequence in their action on bone formation.

THE LYMPHO-EPITHELIAL ELEMENTS

The tonsil and the thymus both represent an interaction in which entodermal activity induces secondarily a local or immigrant accumulation of lymphocytes.

These are so often absent or developed so variously in position, and this applies particularly to the thymus, that no phylogenetic significance can be attached to them.

All experimental evidence, whether derived from high or low vertebrates, fails to attach any specific function to them. The very behaviour of the entoderm, viz. its tendency to form Hassall's corpuscles (and these are found occasionally in the tonsil) does not afford any ground for expecting secretory activity.

The rôle of the lymphocyte is obscure. In allocating a defensive reaction to them the behaviour of lymphatic endothelium and of the reticulo-endothelial elements associated with them is often forgotten. It would seem that all lymphocytes, whether belonging to the haemolymph system or to the lymphoglandular or the epithelio-lymphatic, are the same morphologically and physiologically. They do not put out pseudopodia, they are not phagocytic, they contain no special granules and liberate no ferments as do the granular cells of

the blood. On the other hand, they are all extremely sensitive to irradiation and respond quickly to slight changes such as dietetic defects. They can accumulate in any organ of the body that undergoes atrophy or regression even if this be the usual fate of the organ. They, of course, accumulate at the periphery of carcinomata, disappearing where they have been surrounded by the growth. In experimental transplantation lymphocytes accumulate about the transplant if its constitution is the same or very nearly the same as that of the host. On the other hand, if the two be widely different then the polymorphonuclears accumulate.

They are generally supposed to be protective and resistive, but the evidence seems equivocal and might just as well be interpreted in the opposite sense. Morphologically, being composed almost entirely of nucleoproteid, they resemble in this the heads of the sperm. That they might produce trephones or growth-promoting substances was suggested by Carrel. By analogy it might be suggested that they promote cell division. At present nothing is really known about their functions.

SUMMARY

We have attempted in this essay to set forth briefly a theory of development which is founded on the study of comparative embryology. Such comparative studies have shown that the vertebrate body is laid down in three growth centres which succeed each other both chronologically and topographically. The antecedent centre is in general dominant over and exerts an inducing or organising effect on the region which follows it.

The pharyngeal entoderm lies at the junction of the first and second growth centres and it plays a large rôle in the induction process. This is the real meaning of the complicated series of changes that it undergoes. It is suggested that it functions as an organising region in the early stages of development, and that this may be the meaning of some of the structures developed there which have only a transient existence. Its functions in the later stages of development are made manifest by the ductless glands which arise from it.

Since the pituitary belongs to the first growth centre and the thyroid to the second it follows from this theory of the mode of growth of the embryo that the pituitary should be dominant over the thyroid. The evidence obtained from the comparative physiology of the ductless glands is used to show that this is so, and that the thyroid fails to function without the co-operation of the pituitary.

The allotment of such an important part in development to the pharyngeal entoderm endows its cells with special potencies, and in the course of this essay the manifestation of these potencies on the development of the neck, on the transformations of the neural crest in the hind-brain region, and on the pathology of the growths of this region and the behaviour of aberrant tissues are mentioned.

REFERENCES

ALLEN, B. (1929). "The influence of the thyroid gland and hypophysis on the growth and development of amphibian larvae." *Quart. J. Biol.* vol. IV, p. 325.

BRACHET, A. (1921). *Traité d'Embryologie des Vertébrés.* Masson, Paris.

—— (1923). "Recherches sur les localisations germinales et leurs propriétés ontogénétiques dans l'œuf de *Rana fusca.*" *Arch. de Biol.* t. XXXIII, p. 79.

—— (1927). "The localisation of developmental factors." *Quart. J. Biol.* vol. II, p. 204.

CARREL, A. (1923). "Trephones leucocytaires et leur origine." *Comp. Rend. de Soc. Biol.* t. II, p. 1266.

CHILD, C. M. (1925). "Cephalo-caudal development." *Anat. Record,* vol. XXXI, p. 369.

DE BEER, G. R. (1923–4). "The evolution of the pituitary." *Brit. J. Exp. Biol.* vol. I, p. 271.

DOTT, N. H. and FRASER, J. (1923). "The influence of experimental pituitary and thyroid derangements upon the developmental growth of bone." *Quart. J. Exp. Phys.* vol. XIII.

FRAZER, J. E. (1922). "The early formations of the middle ear and Eustachian tube." *J. Anat.* vol. LVII, p. 18.

—— (1923). "The nomenclature of diseased states caused by certain vestigial structures in the neck." *Brit. J. Surg.* vol. XI, p. 131.

—— (1926). "The disappearance of the precervical sinus." *J. Anat.* vol. LXI.

GASKELL, W. H. (1908). *The Origin of the Vertebrates.* Longmans, London.

GLEY, E. (1914). *Les Sécrétions Internes.* Paris.

GROSSER, O. (1910). "Zur Kenntnis des ultimobranchialen Körpers beim Menschen." *Anat. Anzeig.* Bd. XXXVII, S. 337.

GUDERNATSCH, J. (1926). "Die Spielweite der inneren Sekretion." *Zeitschr. f. Anat. und Entwicklungsgesch.* Bd. LXXX, S. 750.

HAMMAR, J. A. (1909–10). "Funfzig Jahre Thymusforschung." *Ergeb. der Anat. und Entwick.* Bd. XIX.

—— (1921). "The new views on the morphology of the thymus gland and their bearing on the problem of the function of the thymus." *Endocrinology,* vol. V, p. 543.

HOGBEN, L. (1927). *Comparative Physiology of Internal Secretion.* London.

HOGBEN, L. and CREW, F. A. E. (1924). "Studies in internal secretion." *Brit. J. Exp. Biol.* vol. I, p. 1.

JUBA, A. and MIKALIK, P. (1929). "Ueber die Entstehung der Hassall'schen Körpchen." *Zeitschr. f. d. ges. Anat.* Bd. XC, S. 278.

HAMMETT, F. S. (1929). "Thyroid and differential development." *Endokrinologie,* Bd. IV, S. 85.

—— (1929). "Thyroid gland and growth." *Quart. J. Biol.* vol. IV.

KEITH, A. (1929). "The evolution of the human race." *J. Roy. Anthrop. Inst.* vol. LVIII, p. 351.

KINGSBURY, D. W. (1924–5). "Regressive structures and the lymphocyte." *Anat. Record,* vol. XXIX.

—— (1928). "On the nature and the significance of Hassall's corpuscles." *Anat. Record,* vol. XXXVIII, p. 141.

—— (1915). "The development of the human pharynx." *Amer. J. Anat.* vol. XIII, p. 329.

KRANICHFELD, H. (1914). "Einige Beobachtungen welche Annahme einer physiologischen Bedeutung der Schlundtaschen bei den Embryonen der höheren Wirbeltiere nahe legen." *Anat. Hefte,* Bd. L, SS. 1–95.

KRAUS, E. J. (1929). "Zur Frage der Funktion endokriner Organe in der Foetalzeit." *Endokrinologie,* Bd. V, S. 133.

LAUBMANN, W. (1926). "Die Entwicklung der Hypophyse bei Hypogeophisrost." *Zeitschr. f. d. ges. Anat.* Abt. 1, Bd. LXXX, S. 79.

MOATTI, L. (1929). *Les Fistules et les Kystes Congénitaux de la Région Latérale du Cou.* Arnette, Paris.

PENDE, N. (1915). "Ueber eine neue Drüse mit innerer Sekretion (Gland. insularis cervicalis)." *Arch. f. mikrosk. Anat.* Bd. LXXXVI, S. 193.

POPOFF, N. W. (1927). "The histogenesis of the thymus as shown by tissue culture." *Arch. f. Zellforschung,* Bd. IV, S. 395.

SPEMANN, H. (1923). *Zur Theorie der tierischen Entwicklung.* Rectoratrede, Freiburg.

—— (1918). "Ueber die Determination der ersten Organanlage des Amphibienembryo." *Arch. f. Entwicklungsmechanik,* Bd. XLIII.

SPEMANN, H. (1924–5). "Some factors in animal development." *Brit. J. Exp. Biol.* vol. II, p. 493.

RUMPH, P. and SMITH, P. F. (1926). "The first occurrence of secretory products and of a specific structural differentiation in the thyroid and anterior pituitary during the development of the pig foetus." *Anat. Record*, vol. XXXIII, p. 289.

SMITH, P. E. and ENGLE, E. F. (1927). "Experimental evidence regarding the rôle of the anterior lobe of the pituitary in the development and regulation of the genital system." *Amer. J. Anat.* vol. XL, p. 159.

THURNER, W. (1924). "Ueber den Einfluss von Thymusextrakten." *Arch. f. Phys.* Bd. CCII, S. 467.

WILLIAMSON, G. S., PEARSE, I. H. and CUNNINGTON, H. M. (1928). "The two products of thyroid activity." *J. Path. and Bact.* vol. XXXI, p. 255.

DE WINIWATER, H. (1923). "Histologie du corps branchial ultime." *Comp. Rend. de Soc. Biol.* t. II, p. 957.

ABDOMINAL PREGNANCY IN A CAT

By RICHARD H. HUNTER, M.D., M.Ch., Ph.D.
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On opening the abdominal cavity of a cat, which had been killed on account of its "strange behaviour," a number of firm irregularly shaped tumour-like masses were seen attached to the omentum and the intestinal wall. On closer examination a foetal kitten of about full term was also seen, but in a condition of advanced autolysis, and lying free within the peritoneal cavity there were found a number of foetal bones. An X-ray photograph showed that each tumour-like mass contained a foetal kitten, and that this therefore was a case of abdominal pregnancy in a cat.

Cases of abdominal pregnancy in animals are uncommon, and in the literature at my disposal I have been able to find only some half dozen cases in all. Some of them have been stated to be cases of true primary extra-uterine implantation, but though this has recently been stated to occur in the rodents, the evidence produced in support of the cases I have studied appears to be inconclusive and I am more inclined to believe them to be cases in which the foetal sacs have been extruded through a ruptured uterus; and the attachments which are described seem to me to be secondary aseptic inflammatory attachments to the peritoneal surfaces, that is, in none of the cases has there been a clear demonstration of an active placental connection. It is well known that rupture of the uterus does occur in animals. It is commonly due to mal-presentation or to the impaction of a foetus which is too large to pass through the genital passages. In such cases the mother may recover, and after a few months the uterus may show no obvious scar. Simmons was unable to find any cicatrix in the genital canal of a ewe twelve months after such a rupture. Without a most careful examination, both macroscopical and microscopical, a former rupture of the uterus may easily be overlooked and erroneous conclusions drawn.

In the present case studies were first made to decide the presence or absence of a scar.

The specimen as a whole was fixed and hardened in 10 per cent. formalin, and the uterus was then isolated. The blood vessels of both right and left horns were markedly congested. The right uterine horn showed nothing abnormal, but on the left side there was seen, close to the cervical portion, a well-marked "kink." It is difficult to see how this kink could cause an obstruction to the passage of the foetal kittens, as it did not appear to be bound down by fibrous or other bands; but closer examination revealed an area immediately above it,

on the anti-mesenteric border which to the naked eye presented the characters of a cicatrix. This area was cut out and sectioned for microscopic study, and this showed at one place that the continuity of the mucous and muscular walls was broken. Strands of fibrous tissue repaired the break. There is, therefore, in this specimen direct evidence of the rupture of the uterus, and it must be concluded that the foetuses found in the peritoneal cavity were extruded through this rupture, and that the foetal sacs had formed secondary peritoneal attachments.

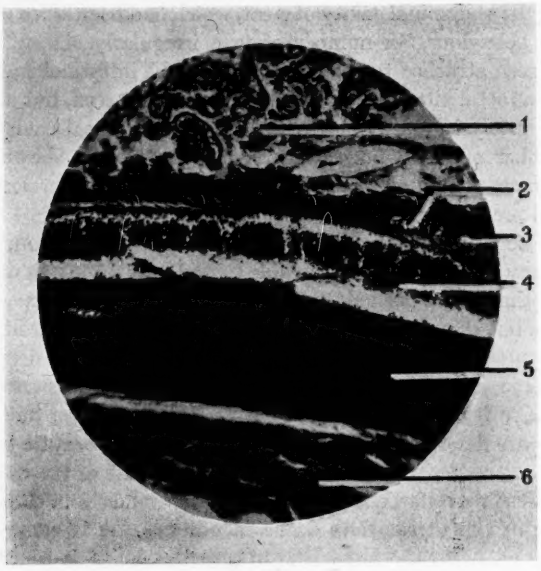

Fig. 1. Microscopic section through the zone of attachment of the foetal membranes to the gut wall. 1. Placental tissue. 2. Foetal blood vessel. 3. Longitudinal muscle layer. 4. Maternal blood vessel. 5. Circular muscle layer. 6. Gut mucosa.

In each mass the foetus was closely surrounded by the foetal membranes, on the outer surface of which was a dense layer of adventitious fibrinous deposit. The external surface of the sacs to the naked eye appeared to be devoid of blood vessels. None of these sacs contained any free fluid, and the body of each foetus was in close contact with the wall of its sac. The fibrinous exudate would be formed as part of the inflammatory reaction on the expulsion of the foetal sacs through the ruptured uterus. The presence of the foetal sacs alone within the peritoneal cavity would be sufficient to bring about the formation of this exudate. Leopold first showed this to be true by placing fresh foetal sacs in the peritoneal cavity of a rabbit, and in 8 to 9 weeks they were covered with this fibrinous exudate. The amniotic fluid was also absorbed.

Each foetal sac was attached to the gut wall at one side only; the remainder of the zonular placenta had entirely disappeared. Sections of the attached region were prepared to study the details of the attachment. A microscopic section through the zone of attachment (fig. 1) shows the mucous layer of the gut wall, the muscularis mucosa greatly developed, the circular longitudinal layer of muscle fibres and beyond the last layer true placental tissue. Between the two layers of the circular and longitudinal muscle tissue lies a dilated blood vessel, cut in its long axis, and containing free blood. The placental tissue lies against the outer surface of the longitudinal muscle layer and the remains of the peritoneal covering can be seen. Within the outer layer of muscle tissue are seen small blood vessels. The histological structure of the wall surrounding these vessels is placental epithelium and shows them to be foetal in origin. It must be concluded therefore that there has been an active invasion of the gut wall by the placental tissue. The blood vessels of the foetal system contain blood cells, and in them there is no trace of thrombosis. There is therefore a healthy foetal blood stream within these vessels.

The relations of the foetal and maternal tissues in this specimen are the same as those normally found between the uterine wall and the placenta of carnivores, where the trophoblast penetrates the uterine mucosa after the destruction of the superficial epithelium, with apposition of the foetal and maternal vessels, without any opening of the maternal vessels or the formation of blood sinuses.

REFERENCES

Bland-Sutton (1904). *The Lancet*, p. 1625.

—— (1896). *Surgical Diseases of the Ovaries and Fallopian Tubes, including Tubal Pregnancies*, p. 319. London.

Boycott (1909). *J. Path. and Bact.* vol. xiv, p. 156.

Galton. *Royal Dick Coll. Mag.* vol. i, quoted by Marshall, 1922, in *Phys. of Reprod.* p. 137.

Goodpasture (1916). *J. Med. Research*, vol. xxxiv, p. 359.

Kuntz (1922). *Anat. Record*, vol. xxiii, p. 237.

Leopold (1881). *Arch. f. Gynäk.* Bd. xviii, S. 53.

Pembrey and Smith (1904). *J. Obst. and Gynaec.* vol. vi, p. 203.

Simmons (1842–43). *Trans. Vet. Med. Assoc.* vol. ii, p. 492.

ENCEPHALOCELE IN THE NASAL REGION

By Dr SHAFIK ABD-EL-MALEK
Egyptian University, Cairo

The case here described is of interest on account of the comparative rarity of congenital tumours in the nasal region and also because the anatomical conditions appear to indicate the site of origin of the abnormal growth.

The subject (fig. 1) is a male child who lived some days and was of normal size and weight. The tumour, which is pear-shaped and is about 5 cm. in length and 3 cm. in breadth at its upper and widest part, is situated in the middle line of the face reaching from the middle of the forehead to the upper lip, and is attached to the head close to the root of the nose. It is covered by normal skin and feels firm to the touch except at two spots, where it is softer and bulges slightly outwards. On the lower surface of the tumour there are two semilunar pocket-like depressions situated on the right and left sides, the former measuring

Fig. 1.

about 5 mm. and the latter 2 mm. in depth. The external nares are visible on each side at the lower and lateral part of the tumour. The right opening is rounded and larger than that on the left side, which is oval in shape.

On removal of the skin over the tumour a sparse layer of muscle fibres was found at the upper and lateral parts, probably representing portions of the frontalis and orbicularis muscles. Beneath the muscle was a strong fibrous layer which was traced backwards until it became firmly attached to a cartilaginous tube which is described below. On opening this fibrous sheath a mass of whitish material of a soft texture was seen which could be separated quite easily from the sheath, and which to the naked eye looked like brain tissue.

A sagittal section (fig. 2) was made through the middle line of the head

including the tumour, and it was then possible to see the connection of the latter with the internal parts. The contained mass was found to enclose two cavities, and to be continuous with a similar but slightly lighter coloured tissue closely resembling brain substance passing into the cranial cavity and situated above the nasal septum and below the nasal bones. This is ensheathed by a strong fibrous covering which could not be separated into layers. It is smooth and glossy on the surface next the brain-like material, but its outer surface is rough and attached to the surrounding cartilaginous tube by shreds of fibrous tissue. This fibrous sheath is continuous posteriorly with the dura mater covering the under surface of the frontal lobes of the brain. The cartilaginous tube (fig. 2) when seen in section consists of two horizontal plates of which the upper is thicker and more irregular than the lower. Above this plate

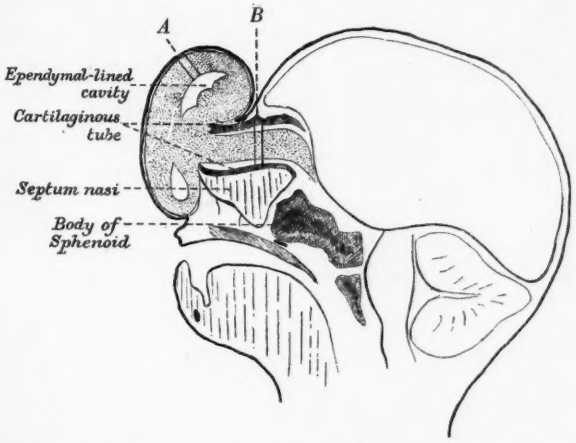

Fig. 2. Mesial sagittal section through head, showing connection of tumour with internal parts. *A* and *B* indicate sites from which the sections were taken.

the partially ossified nasal bones can be seen. The lower plate is in relation in the middle line with the nasal septum which has been pushed down. The tube commences anteriorly at the site of attachment of the tumour, into which it appears to project, and ends posteriorly at the lower part of the frontal lobes of the brain and in close relation to the upper part of the body of the sphenoid bone.

The section passed to the left side of the nasal septum, which is therefore intact. It is triangular in shape, the base being in relation to the body of the sphenoid, while the upper border which is nearly horizontal extends the whole length of the under surface of the cartilaginous tube as mentioned above. The lower border extends from the anterior end of the upper border downwards and backwards to meet the base at the body of the sphenoid. The latter, with

the septum, has been so much depressed by the growth of the tumour that it lies only 2 mm. above the hard palate, reducing therefore the posterior nares to two slit-like apertures 2 mm. in height and about 6 mm. in breadth. At the same time the vomerine part of the septum appears to be absent and the vomer is represented by a spicule of bone which occupies the interval between the body of the sphenoid and the hard palate (fig. 2).

In order that the relation of the cartilaginous tube might be better seen, the left eyeball was removed and a coronal section made through the middle of the tube including the medial wall of the orbit, of which it was found to form the upper part (fig. 3).

By raising the frontal lobe on the left side it was possible to follow the course of the tumour substance, which when traced posteriorly divided into two delicate strands, each of which passed backwards and laterally to join the olfactory bulb of the corresponding side on its medial aspect (fig. 4).

It would seem therefore that the tumour had its origin primarily from the developing olfactory bulbs, and growing forwards pushed the nasal bones, nasal

Fig. 3.

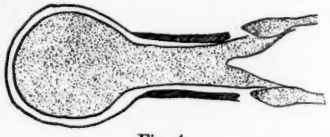

Fig. 4.

Fig. 3. Section through left orbit, showing cartilaginous tube forming part of medial wall of orbit.

Fig. 4. Semi-diagrammatic drawing showing connection of tumour with olfactory bulbs.

process of the frontal bone and crista galli upwards and the lamina perpendicularis of the ethmoid and body of the sphenoid bone downwards, the latter being depressed almost to the level of the hard palate and reducing the posterior nares, as mentioned above, to mere slits.

Sections taken from the tumour itself and from its pedicle were examined microscopically by Dr M. F. Sorour.

Fig. 5 is a semi-diagrammatic drawing made from the section through the tumour and shows beneath normal skin a layer of fibrous tissue, embedded in the upper layer of which are many sebaceous glands, sweat glands and hair follicles. This encloses the tumour substance proper, which appears to be a typical glioma consisting of spindle-shaped triangular cells as well as rounded cells with many protoplasmic processes. It is vascular and the vessels are thin-walled, consisting of an endothelial layer resting directly on the tumour substance. It enclosed a cavity, part of which is included in the section and is lined by ependymal cells.

Fig. 6. This section was taken through the pedicle and exhibits from without inwards the following layers:

(*a*) A plate of typical hyaline cartilage.

(*b*) A fibrous sheath which is very vascular.

(*c*) Normal-looking brain tissue with large pyramidal cells.

Also tubular structures lined with high cubical epithelium (ependyma) traversing the nervous tissue.

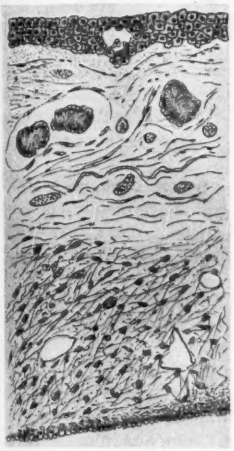

Fig. 5.

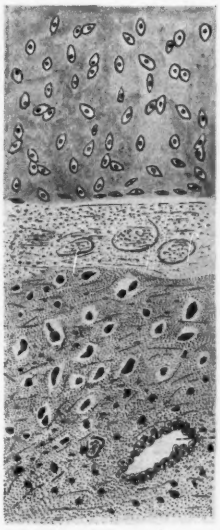

Fig. 6.

CONCLUSIONS

The tumour is of cerebral origin and appears to be a protrusion from that part of the neural tube which gave rise to the olfactory bulb. This is indicated both by its connection with the latter and by the ependymal-lined cavities contained in it.

The cordial thanks and gratitude of the writer are due to Prof. D. E. Derry for his kind and invaluable help in producing the paper; to Dr M. F. Sorour for the microscopic examination of the sections, and to Dr B. Boulgakow for his assistance in preparing the drawings.

ANATOMICAL NOTE

PERSISTENT "LEFT SUPERIOR VENA CAVA," "LEFT DUCT OF CUVIER" AND "LEFT HORN OF THE SINUS VENOSUS"

By BHUPENDRA NATH BASU, M.B.

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THE presence of two superior venae cavae is of rare occurrence in adult life. During my experience of the last 14 years in the Anatomical Department of the Carmichael Medical College, Calcutta, while more than 600 bodies have been dissected, I have not come across a condition similar to that noted in this case; although one gathers from the literature that it is not an uncommon abnormal condition.

The specimen under review was dissected out from a subject brought to this department from the hospital. The records of the case showed that the subject was a Hindu male, aged 42 years. His body was thinly built and he was a day labourer by occupation. He died of pneumonia, and during dissection of his body the following abnormal conditions of the cardio-vascular systems were found:

(1) The heart and the great veins very much dilated and filled up with blood clot.

(2) The presence of two superior venae cavae opening separately into the right atrium of the heart.

(3) Absence of the transverse portion of the left innominate vein.

(4) Absence of the coronary sinus.

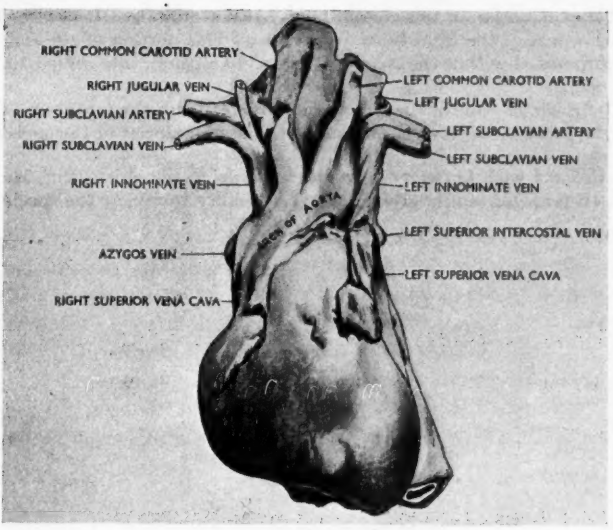
(5) The union of the left superior vena cava with the inferior vena cava before opening into the lower part of the right atrium of the heart by means of a common opening.

The right innominate vein is formed by the union of the right jugular and the right subclavian veins. It leads downwards and continues as the right superior vena cava which opens at the upper and back part of the right atrium of the heart. The left innominate vein begins by the junction of the left jugular and the left subclavian veins and passes vertically downwards into the left side of the superior mediastinum, crossing in its course the arch of the aorta and the structures forming the root of the left lung. It is continued as the left superior vena cava behind the left auricula and on the dorsal aspect of the heart, occupying the coronary sulcus. While situated on the dorsal side of the heart it receives all the tributaries of the adult coronary sinus (cardiac veins). Finally, it unites with the inferior vena cava just before they pass into the lower and back part of the right atrium of the heart through a common opening. The left superior intercostal vein joins with the left superior vena cava before the latter passes into the back of the heart.

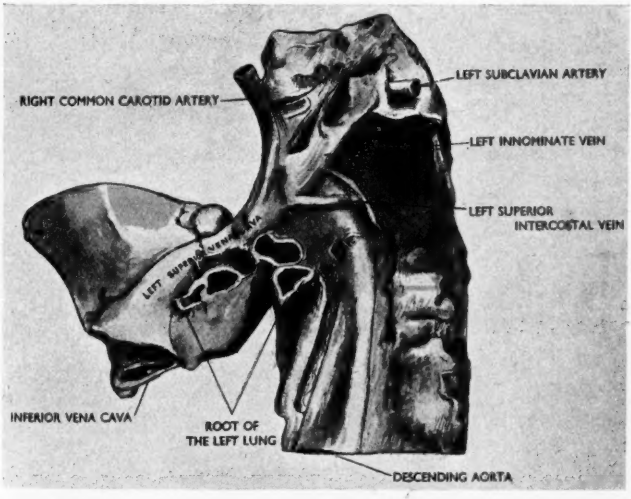
Embryological explanation

This is a case of rare developmental anomaly in which the left anterior cardinal vein and the left duct of Cuvier, of foetal life, are remaining patent throughout their whole course and are persisting as a single vein which is characterised as the left superior vena cava. The right superior vena cava is present in this case, but the transverse anastomosis which usually forms the left innominate vein is entirely absent.

This condition is normal in some Mammals, but it is very rare in Man.



Anterior view.



Posterior view.

The peculiarity of this particular specimen is that the left superior vena cava is joined with the inferior vena cava to form a common trunk before they pass into the lower and back part of the right atrium of the heart through a common opening. The only possible explanation of this condition is that not only the left horn of the sinus venosus but a part of the right horn also persists. This portion of the right horn is usually incorporated within the cavity of the right atrium, into which the inferior vena cava opens.

The two drawings—one of the anterior and the other of the posterior view of the heart and the great blood vessels—illustrate the abnormalities of the specimen.

In conclusion I wish to express my warm thanks to my chief, Prof. M. N. Bose, M.B., C.M. (Edin.), for kindly giving me every facility to review the specimen.

RECENT APPOINTMENTS

At a meeting of the Senate of the University of London (18 November, 1931), Dr H. A. Harris was promoted to the professoriate of the University. Dr Harris's promotion gives the Department of Anatomy and Embryology in University College a third Chair in Anatomy—Prof. G. Elliot Smith, F.R.S., being Director of the Department, while Prof. J. P. Hill, F.R.S., holds the Chair of Embryology. Dr Harris becomes Professor of Clinical Anatomy, continuing his work in offices he already holds at University College and University College Hospital. Dr Harris's various researches are well known to readers of this *Journal.*

Dr Robert D. Lockhart, M.B., B.Ch. (Aberd.), Lecturer in Anatomy in the University of Aberdeen, has been appointed to the Chair of Anatomy in the University of Birmingham, in succession to Prof. J. C. Brash. Dr Lockhart is 37 years of age, has been teaching and researching in Aberdeen for twelve years. His chief publications refer to neurological and anthropological subjects.

REVIEWS

The Evolution of the Nervous System in Invertebrates, Vertebrates and Man. By C. U. Ariëns Kappers, M.D. Amsterdam, D.Sc. Yale University. (Haarlem: De Erven F. Bohn.) 1929. Pp. 335. 146 illustrations. Price: in loose cover, 8.75 fr.; bound, 10 fr.

Prof. Ariëns Kappers is already well known in this country, for his lectures have reached wide and appreciative audiences and his writings are indispensable to all those who would arrive at a comprehensive understanding of the nervous system. His magnificent work on the comparative anatomy of the nervous system proclaimed him one of the foremost anatomists of his time, and the present work affirms this distinction. This volume on the *Evolution of the Nervous System* is in part an epitome of his great work, but also supplements that work by giving us an understanding of the nervous system of the invertebrates and carrying us through the anatomy of the brain in the fossil and recent races of Man.

The opening paragraphs present the author's view, in a succinct manner, of the relation of function and structure. The functions brought to high perfection in the nervous system—reception, conduction, correlation, and elaboration of impulses—are also the properties of protoplasm, for they are present in the lowest forms of life where there is no nervous system. Even memory, attention and association, generally considered as functions peculiar to the mental life, are adumbrated in the primordial protoplasm. Memory might be regarded as the organic principle of heredity; attention is concentration on one function to the exclusion of all others, and has its organic counterpart in the process of tissue differentiation; while association is the mental form of organic correlation expressed in the nervous system by neurobiotaxis.

The review of the principal classes of invertebrates is most skilfully presented in order to illustrate some of the fundamental laws that govern the form and function of the nervous system. Neurosensory epithelium represents the first kind of nerve tissue evolved. This variety possesses neurofibrillae but no Nissl granules, and its single undifferentiated neurite invades the subjacent tissue. In this subjacent tissue it will usually come into relationship with the next order of differentiation in nervous tissue, the primitive ganglion cell, which has both neurofibrillae and Nissl granules but whose processes arise from any part of the cell body and show no differentiation into axis cylinder or dendrites. The third variety, the neurone with its synaptic relationship to other neurones, first appears in worms. Kappers has drawn the very interesting conclusion that the neurone type is not a differentiation from the primitive ganglion cell but a return to and a differentiation from the neurosensory cell. The neurosensory cells are to be found in the eye and ear and elsewhere in the vertebrate, while the enteric plexuses represent in them the primitive ganglion cells.

Among the Echinoderms neurosensory cells and primitive ganglion cells occur superficially, but also a deeper radially arranged nervous system which is divisible into an ectoneural and a hyponeural system. These latter, though perhaps physiologically combined, appear to be morphologically distinct. The hyponeural system arises from the coelom and is not ectodermal in origin.

In worms the nervous system is composed of two parts, a superficial, containing neurosensory cells, and a deep, containing primitive ganglion cells, and a central nervous system with head ganglia and nerve cords dorsally and ventrally. All parts of the nervous system arise from the ectoderm. Indeed the ectoderm may be regarded as a generalised nerve tissue, while the nervous system proper derived from it is but a more specialised system. The tendency for the nervous tissue to concentrate in

regions where stimuli are most abundant, the ability of the cell body, drawing its neurite after it, to migrate towards the source of the stimulus—these are the phenomena of Kappers' law of neurobiotaxis. The development of the head ganglia depends very little on ascending sensory fibres, for these are very few, but depends mainly on the concentration of sense organs in the head region. The concentration of nerve tissue in the head region and the presence of dorsal and ventral nerve cords are examples of nerve tissue arranging themselves neurobiotactically in accordance with the receptive activity and the co-ordinative needs of the body. The cephalisation of nerve tissue, which depends on the abundance of afferent impulses in the head region, is to be regarded as the primary cephalisation. The cephalisation that arises with the associative functions comes later and is secondary, and is of course to be found in the higher invertebrates, like the insects, and of course in the vertebrates.

In the Molluscs a partial decussation of the fibre paths connected with the static organs so that the lateral half of one combines with the medial half of the other, occurs and parallels exactly what occurs in the optic chiasma of the highest vertebrates. This again is in accordance with the law of neurobiotaxis. The arrangement of the eye in the Molluscs brings out another principle. The eye and its associated ganglia at this level originate separately from the ectoderm, but the eye proper contains only the percipient elements, while the rest of the elements characteristic of the vertebrate retina are contained in the optic lobe. This illustrates how all the elements of a vertebrate eye are present in the mollusc but are scattered, while with higher organisation they become more and more concentrated. This same principle is illustrated in the efferent system, for the muscles receive their innervation from the peripheral ganglia and the arrangement resembles the autonomic system of vertebrates. Greater and greater concentration is a principle of the evolution of the nervous system.

The concentration of nerve tissue ganglia in the thoracic region of the insect is another example of neurobiotaxis, for in this region the organs of locomotion are situated and the outgoing impulses are most abundant. In the insects a new advance is registered, for the innervation of the skin is now abundantly dependent on the dendrites of bipolar ganglion cells and the ectodermal cells have become specialised, so that they afford a lowering of the threshold for certain kinds of stimuli. Thus we pass from more general dyscritic sensations to the epicritic discriminative kind. Nerve roots are present in the arthropods but arranged orally and caudally. These contain both sensory and motor fibres. However, pure motor roots and pure sensory roots do occur, but the ventral is sensory and the dorsal motor. This again, in the opinion of Kappers, is an example of neurobiotaxis, for the ventral, being nearer the skin, accordingly subserve sensory functions.

In those insects with highly correlated behaviour the corpora pedunculata are the most characteristic structures in the cerebral ganglia. These are highly developed in worker bees, in socially living wasps, and other related forms. When removed, some instincts, such as courting and the care of eggs, are disturbed. The protocerebrum in which these corpora pedunculata lie is entirely optic and associative in character, a further example of the part played by vision in evolution. It is the compound facet eyes that are associated with the corpora pedunculata and not the ocelli.

From the invertebrates the author passes to the Enteropneusts, and from the description it appears that in these we have both the vertebrate and the invertebrate types of nervous system intertwined. The medullary tube that develops in these Hemichordata seems undoubtedly the forerunner of the vertebrate system.

The description of the nervous system of *Amphioxus* persuades us to believe that this prototype is really an aberrant and degenerate form, a conclusion to which some embryologists have come on quite other grounds.

The account of the nervous system in the vertebrates covers ground already made familiar in some degree by the two great volumes on the comparative anatomy of the nervous system already referred to. Amongst many cogent examples of the

operation of the law of neurobiotaxis one is given in the chapter on the phylogeny of the autonomic nervous system that seems interesting. It is the opinion of the author that it is the predominance of axon reflexes in the autonomic system that has led to the migration of its ganglia towards the periphery. The functional and morphological division of the nervous system comprised in the formation of the basal and alar lamina is used to determine the functions of the various parts of the diencephalon. A very clear account is given of the subdivisions and homologies of the various parts of the basal ganglia in the different orders of vertebrates, and the fourfold subdivision of the telencephalon is made manifest in the same orders. Indeed the account of the vertebrate nervous system, most stimulating and instructive as it is, makes it clear how much more work is necessary before we have a clear comprehension of its many problems.

The primordium neopallii Kappers accepts as appearing first in the reptilian brain, and starting from here there follows an elucidation of the neocortex of mammals and the significance of its stratification and fissuration. This naturally leads on to the study of the cerebrum in fossil and recent varieties of Man.

This anthropological section, the author says, he included in order to stimulate research in this field. His presentation of the problems involved, the methods available, and the work that has been done make this an admirable starting-point for anyone entering this field of research.

In this brief note it has been our intention to indicate in some little way the attitude of the author to the evolution of the nervous system, his insight into the functional and structural mechanisms involved, and the further work he would like to see undertaken in these fields by anatomists.

We regard the book as a splendid piece of work by a great anatomist.

It is a pleasure to observe that the book is dedicated to Dr Elizabeth Crosby, of Ann Arbor University, Michigan, who has done splendid work in comparative neurology.

Four Lectures on "Abnormal Conditions of the Teeth of Animals in their Relationship to Similar Conditions in Man." By Sir FRANK COLYER, K.B.E., F.R.C.S., L.D.S. (The Dental Board of the United Kingdom, 44, Hallum Street, London, W.1.). Pp. xi + 167. 217 figs. Price 5*s*. net.

This work, written in a lucid style, is largely a description of specimens of which excellent photographic reproductions are included. Many interesting facts of a general anatomical and clinical nature are brought out. The first lecture deals with injuries of the jaws and teeth, and one is led to wonder why osseous union does not occur in repair of the fractured maxilla, while interesting specimens of repair in teeth are described and the possible relationship of this to the development of a composite odontome is suggested. The difference of repair in teeth of persistent growth and of limited growth is indicated. Lecture II, on "Caries of the Teeth," deals with variations in this disease amongst wild animals in their natural state or in captivity, and in domestic animals. The figures show that the old world monkeys are more susceptible to caries both in their wild and captive state, although with both there is more caries in the latter than in the former. Lecture III deals with the progressive destruction of the tooth sockets under the same three headings, and there is throughout the close relationship of this to the physical character of the food as distinct from its chemical character. Lecture IV is perhaps of most anatomical interest, being on "Positional Variations of the Teeth." The writer shows that such variations are not limited to Man but are relatively common not only in the anthropoid apes but also in the old world monkeys in the wild state with a lesser number in the new world monkeys. There is also a great increase in the percentage of all

groups in the captive state. The aetiology of these positional variations raises important problems of evolution and heredity, as well of alterations which are the result of environment during growth. The book will make a useful addition to the reference library of the anatomical department. There is no index but a very complete contents table which makes up for this defect.

The Aetiology of Irregularity and Malocclusion of the Teeth. By Prof. J. C. Brash, M.C., M.A., M.D. With an appendix "Concerning Orthodontic Problems and their Solution" by Miss M. L. Tildesley. (The Dental Board of the United Kingdom, 44, Hallum Street, London, W. 1.) Pp. xi + 274. 193 figs. Price 5*s.* net.

This work consists of four lectures given by the author under the auspices of the publishers. In it he has reviewed "the evidence of the participation of probable factors" by abstracting the literature on the subject in the light of his own studies of the growth of the alveolar processes of the jaws. The main conclusion at which he arrives is that we are not yet in a position to come to any conclusion, although he leans towards the attitude that irregularity and malocclusion of the teeth are extended conditions, and suggests that this should be the provisional hypothesis from which to start future investigations.

In the first lecture, after an historical survey and a definition of the general nature of the problem, he passes on to consider the incidence of these irregularities. The second lecture deals with the problems of heredity, while the last two are concerned with the physiological and pathological conditions of growth. These four lectures contain a wealth of material abstracted from a large number of references, from which a full index is wanting. Its place is taken neither by the detailed contents nor by the full bibliography. Prof. Brash keeps his own work modestly in the background. The appendix is a thoughtful essay by Miss M. L. Tildesley of the Royal College of Surgeons Museum, in which she urges the value of and need for statistical evidence properly collected and applied. The books bring up to date the knowledge of the subject. The results are largely negative, but are equivalent to a thorough clearance of the ground on which another superstructure may be raised.

A Manual of Embryology. The Development of the Human Body. By J. Ernest Frazer, F.R.C.S. Eng., Professor of Anatomy in the University of London; Lecturer at the Medical School of St Mary's Hospital, etc. (London: Baillière, Tindall and Cox.) 1931. Pp. viii + 486. 282 figs. Price 30*s.* net.

It becomes manifest as one reads this original work that Prof. Frazer took real pleasure in writing it. For over a quarter of a century he has been continuously busy with his microtome and microscope investigating and reconstructing developmental stages of various vertebrate forms but chiefly human. From time to time he has published in this *Journal* an account of the results of some of his investigations. But what he has published is but a tithe of what he has done. As he wrote the twenty-three chapters which lie within the covers of his book, it must have given him satisfaction to incorporate in its text the numerous original observations which had accumulated in his hands. He must also have welcomed the opportunity of giving expression to his own interpretations of the significance of certain embryological processes, for, as is well known, Prof. Frazer is heterodox in some of his views.

The only part of his task which appears to have troubled him was that of omission of detail. To keep his manual within the limits of a medical student's powers of acquisition he found that "the drastic cutting down of detail is not pleasant," for he believes that it is just a knowledge of detail that will provide a clue to many problems of disease and of pathology. Nevertheless neither student nor teacher will have reason to complain that detail is lacking in either the author's text or in his illustrations. In brief, he has produced a book which has been much needed—a real English manual of human embryology founded on original observation. In this respect Prof. Frazer's book is a pioneer work—the first of its kind to appear in Britain.

The first nine chapters carry the reader from the production of the sex cells to the full development of the embryo and the establishment of a placental circulation. Chapter then succeeds chapter in which the development of the various organs and systems are described—the framework of the trunk—the nervous system and sense organs, the foregut and primitive pharynx, the skull, face and teeth, the circulatory system, the respiratory system, the abdomen and digestive system, the cloaca and genital system and finally the limbs and dermal system. Naturally the parts of the body which the author has investigated come in for the fullest treatment, but this in no way detracts from the balance of his book. It is a complete and systematic exposition of the present state of our knowledge of the human body.

Where references to comparative embryology assist in the interpretation of the development of the human body they are seized upon and duly applied. More especially do we commend Prof. Frazer's physiological attitude of mind; he never forgets that at all stages of development the embryo and all its parts are living and that they have functions to perform. The book is one which is destined to take an abiding place amongst standard text-books and will serve the needs of teacher and of student for many a year to come.

Evolution of the Facial Musculature and Facial Expression. By Ernest Huber, Ph.D., M.D., Associate Professor of Anatomy, the Johns Hopkins University. (Baltimore: Johns Hopkins Press; London: Oxford University Press.) 1931. Pp. 184. 28 figs. Price 13*s.* 6*d.* net.

The evolutionary story of the facial musculature is one of the most interesting of all the revelations of modern biology, and it loses nothing in the way which Prof. Huber has told it. Not that we know all the details of this story, but those who want to know more will do well to take Prof. Huber's book as their guide. Many have contributed to the story so far as it is now known; their contributions are duly recorded by Prof. Huber. Of those who receive mention none deserve it more than Prof. G. Ruge, whose dissections and drawings were well known to those who commenced their investigations some forty years ago. It was from Prof. Ruge that the author of this monograph on the facial musculature drew his inspiration. Had not the author given this information one would have suspected that his enquiries into the anatomy and psychology of the muscles of expression had arisen from a study of expression as manifested in the faces of various races he has had opportunities of studying in life. For beyond a doubt each of the main races of mankind has its own habitual modes of facial expression. Prof. Huber's object is to prove that this difference in expression is reflected in a difference of the arrangement of the facial musculature of each race. Each race has its own neuro-musculature machinery. Prof. Huber saves pages of text and his readers hours of labour by a free use of excellent line drawings.

Prof. Huber emphasises the similarity of the facial musculature of the chimpanzee, gorilla and Man and notes the aberrant development of the facial muscles

of the orang. He cites this evidence, quite rightly, in support of the thesis that Man has come from the same stock as the African anthropoids, and would exclude the orang from the same evolutionary phylum. In this he is probably not justified, for the body of the orang is crowded with systems which show peculiar degenerative changes—its nose, forehead and facial musculature having some of the manifestations of this trend. There is something amiss in the mentality of the orang as well—his sullen, morose, emotional nature being symptomatic of his degenerate nature. Evolutionary changes have carried the orang away from the more primitive and robust African anthropoids, but fundamentally he is of the same stock. The orang is the victim of the action of some hormonal complex—one not unlike that which affects the Mongolian races of mankind and which the late Prof. Klaatsch sought to explain by deriving the Mongol from an orang stock.

We have said enough to give readers an inkling of the service which Prof. Huber has rendered to Anatomy by publishing his graphic and interesting monograph on the facial musculature—the chief servants of Man's emotions and feelings.

Cunningham's Text-Book of Anatomy. Edited by ARTHUR ROBINSON, M.D., F.R.C.S. 6th Edition. (London: Humphrey Milford, Oxford University Press.) 1931. Pp. 1554. 1125 figs. and 2 plates. Price £2. 2*s*. net (English price).

Its continued universal employment by teachers and students is sufficient commendation for this Text-book of Anatomy. The fifth edition appeared in 1923 and the present work is a worthy successor in maintaining the high standard of excellence in the presentation of anatomical facts. All the sections have been revised, rearranged, or rewritten, the better to adapt them for present-day requirements, and important recent work has been incorporated. The beautiful illustrations, a feature of former editions, have been judiciously added to, or in some instances replaced, in every section.

The new contributors are Profs. D. M. Blair (Arthrology), J. C. Brash (Myology), John Fraser (Surface and Surgical Anatomy), J. S. B. Stopford (Peripheral Nervous System), T. Wingate Todd (Respiratory System) and C. M. West (Organs of Sense and the Integument). The remaining sections continue under the authorship of Prof. A. F. Dixon (Urogenital System), Dr E. B. Jamieson (Osteology), Prof. Arthur Robinson (General Embryology; the Vascular System), Prof. G. Elliot Smith (Central Nervous System), Prof. D. Waterston (Digestive System) and Prof. J. T. Wilson (Ductless Glands).

The B.N.A. terminology has been retained, as has also the glossary of B.N.A. and old terminology equivalents. A welcome feature of many sections is the extension of the discussion of general principles of the "system" at the commencement of the section. A notable feature of the section on Arthrology, which has been entirely rewritten and considerably extended, is the replacement of the B.N.A. classification of joints by that recommended by the "Nomenclature Committee" of the Anatomical Society of Great Britain and Ireland. This latter classification is more in accord with present-day teaching. In the classical section on the Central Nervous System many simple line drawings have been added which are very valuable to the student (for instance, when reading the complex connections of the basal ganglia). The customary description of the lower visual centres (in Man) has been replaced by the modern conception, and the structure and connections of the substantia nigra and the connections of the olivary complex have been incorporated. In the Peripheral Nervous System section recent work on the innervation of the teeth, the accessory phrenic nerve, the digital nerves of the hand and the innervation of the blood vessels of the limbs has been added. No mention is made of any branch of the glosso-

pharyngeal nerve to the carotid sinus (the carotid sinus itself being omitted from consideration in the Vascular System section) and the description of the muscular plane of a typical intercostal nerve is not in accord with present-day teaching. (Likewise, in the Myology section, the intercostal musculature has not been brought up to date.)

A useful feature of the Osteology section is the reference tables of the times of appearance of the centres of ossification and of the fusion of the epiphyses in the two sexes. These times have been revised in the light of recent studies (chiefly radiological) of this subject. It is questionable if it is an advantage to relegate the description of the "attachments" to a bone to a relatively short condensed paragraph at the end of the description of the naked eye features of the bone. The inclusion and description of a series of radiograms of the bone ends (joints) at important "growth ages" would be of great value. It is during his anatomical (pre-clinical) studies that a student should learn "normal radiology." In this connection it might be suggested that the two radiograms of the skull at the end of the book would be of enhanced value to the student if they were covered with transparent paper on which tracings and labelled pointers were drawn. Furthermore, radiograms of the heart, lungs, alimentary tract (after opaque meals), and of the ureter and bladder (after injection of opaque fluids) might with advantage be inserted and described in the appropriate sections. In the Ductless Glands section recent work on the structure of the spleen, carotid body and pituitary body have been added. In the section on General Embryology recent views on the transmission of hereditary characters have been incorporated, but the statement that the posterior third of the tongue is developed from the second visceral arches is not in agreement with present views nor with the nerve supply to that part of the organ. In the section on the Vascular System a notable omission is the sinu-auricular node. The description of the "conducting system" of the heart, in view of its clinical importance, might be considerably extended, and illustrations of this system inserted. The blood supply of this system and the age changes in the blood supply of the heart itself might also be incorporated. In the section on the Digestive System attention is drawn to the effect of posture on the position of some of the abdominal viscera. It would be of advantage also to refer to the relation between bodily habitus (stature) and the position and shape of the thoracic and abdominal viscera. It is more important to describe the palatine tonsil at its maximum development, as regards its extent and relation to the supratonsillar fossa and glossopalatine fold. No other clinically important organ is normally described in a state of atrophy. An example of a disadvantage of multiple authorship is seen in the description of "pressure pouches of the oesophagus" in the Digestive System section. These are correctly described as "pulsion diverticula of the pharynx" in the Surgical Anatomy section. It would be well in the three sections (Myology, Digestive System and Surgical Anatomy) to name and define the crico- and thyro-pharyngeus parts of the inferior constrictor muscle.

The criticisms detailed above are of a minor nature and the book will continue to occupy a premier position on the anatomist's bookshelf.

Pocket Atlas of Anatomy. By VICTOR PAUCHET and S. DUPRET. 2nd Edition. (London: H. Milford. Oxford Medical Publications.) 1931. Pp. vi + 377. With 345 illustrations. Price 12*s.* 6*d.* net.

This excellent little atlas was first published in 1928, and was the outcome of M. Pauchet's lectures in anatomy and operative surgery. The second edition is slightly larger than the former one, and contains some twenty-eight new illustrations. The book is exactly what the authors have set out to make it, namely a small atlas

of anatomical plates, accurately drawn, and carefully reproduced. The book will be the welcome friend of students, not only in the dissecting room, but also when examinations are contemplated.

It is of convenient size and weight for the pocket. In this second edition, an index and an alphabetical list of the plates are included; this is certainly an advantage, as it makes for ready reference.

A Short History of Anatomy. By RICHARD H. HUNTER, Ph.D., M.D., M.Ch., Lecturer in Anatomy, Queen's University, Belfast. 2nd Edition. (London: John Bale, Sons and Danielsson, Ltd.) 1931. Pp. 86. Price 3*s.* 6*d.*

We are glad to note that a second edition has been called for of this useful little book. It is a sketch of the history of dissection, rather than of anatomy in the full sense, up to the passing of the Anatomy Act of 1832; in this edition a more extended account is given of the unsavoury episodes which made clamant the need for such legislation. The book does not attempt to cover too much within its compass, but gives a readable account of the salient facts, grouped around the great names in Anatomy. From it the student beginning dissection can obtain, at little cost in time or money, a serviceable historical impression which will lend interest to his present work and may lead him later to deeper reading in the history of Medicine.

On p. 62 the name of Prof. Jeffray of Glasgow is wrongly rendered Heffrey.

Baillière's Synthetic Anatomy. By J. E. CHEESMAN. Part XI. Perineum (Male). Part XII. Perineum (Female). (London: Baillière, Tindall and Cox.) 12 plates in each part. Price 3*s.* each.

With these two parts now issued, this well-known Atlas of transparent plates approaches the end of its course of indefatigable ingenuity. The perineum is portrayed in successive oblique levels from below upwards; the plates of the female perineum are life size, while those of the male present a slightly wider field on a correspondingly reduced scale. It must be confessed that here the ingenuity seems rather to outrun usefulness. The illustrations are less readily interpreted than in other parts of the series, and it is doubtful if many students will put forth the effort needed to unlock the information which is bound up in these plates. The Atlas will be completed by one further part, illustrating the brain.

Researches in Craniometry. By JOHN CAMERON, M.D., D.Sc., F.R.S.E., F.R.S.C., Professor of Anatomy, Dalhousie University, Halifax, N.S. from 1915 to 1930. Vol. II. 1931. A limited edition of this volume has been published by the author.

In his first volume of *Researches* Prof. Cameron included 25 papers—23 of which were devoted to Craniology. The present volume (vol. II) contains 34 papers—all of which—with one exception—contain an account of methods, most of them new, which Prof. Cameron has applied to the study of the skulls of different races of mankind and different genera of anthropoid apes. These studies were originally published in the *Transactions of the Royal Society of Canada* and in the *American Journal of Physical Anthropology.* Prof. Cameron was among the first to discover that the Anatomical Department of Western Reserve University, under the able Directorship of Prof. T. Wingate Todd, is the Mecca for all who wish to make researches on Human Craniology. Only in Prof. Todd's department can the investigator find what he needs—long series of human skulls and skeletons of known age and sex. Most of

Prof. Cameron's researches have been made on Western Reserve material—preserved in the Hamann Museum.

The greater number of the papers published in vol. II relate to that part of the skull which is exposed on mesial section and is bounded by lines which join basion pituitary point (as defined by Prof. Cameron), nasion and alveolar point. Prof. Cameron approaches the problems of the skull as a geometrician rather than as a physiologist or mechanician. By uniting the points just named he marks out three triangles—each of which has its sides duly measured and its angles estimated—in skulls of various human races. The method gives opportunities of comparing human and anthropoid skulls. These studies of the cranio-facial axis lead on to new studies of the face and to an enquiry into the relationship of the auditory passages to total length of skull. Papers of interest relate to "Length-height," "Breadth-height," indices of the cranial cavity in diverse races and to the influence of the sex factor on such indices. The series of studies on the face open new ground. Excellent illustrations are provided and the volume is neatly bound.

Handbuch der Vergleichenden Anatomie der Wirbeltiere. Edited by ERNST GÖPPERT of Marburg, ERICH KALLIUS of Heidelberg, WILHELM LUBOSCH of Würzburg and by the late LOUIS BOLK of Amsterdam. (Berlin: Urban and Schwarzenberg.) 1931. Vol. I. Pp. xvi + 752. 561 figs., with 10 coloured plates. Price: unbound, R.M. 100; bound, R.M. 106.

The volume here announced is the first of a series of six volumes in which representative anatomists, chosen for the greater part from German-speaking universities, are to present Comparative Anatomy in the light of modern knowledge. The publishers hope that the remaining five volumes will appear in quick succession, so that this standard work in the German tongue should be completed in 1934. Those who subscribe for the whole work are privileged to purchase the present volume (vol. I) at a reduced rate.

This work is intended to take the place of Gegenbaur's classical treatise. Thirty years have come and gone since the second volume of Gegenbaur was published, and this new work reflects the revolutionary changes which have overtaken comparative anatomy in the interval. Instead of a single editor five are now needed—really four, since death has removed one of the greatest of them—the late Prof. Bolk—since this immense work was planned. The editors have enlisted sixty anatomists to help them in giving a presentment of the present state of comparative anatomy. The day is not so far distant which will require a thousand men to accomplish the task which has been undertaken by sixty modern anatomists. The growth of knowledge confers penalties as well as privileges on coming generations of students and workers.

Not only has knowledge grown rapidly in the past thirty years but the beliefs which determine lines and methods of research have also changed very greatly. Alterations in point of view are made very evident in the pages of the first volume. One-third (267 pages) is devoted to the exposition of general matters relating to comparative anatomy; two-thirds (484 pages) contain accounts of the anatomy of the integumentary system and of the various specialisations of that system. It is in the "Allgemeiner Teil" we see reflected changes in point of view. The outlook has become more biological. The editor-in-chief, Lubosch of Würzburg, gives a historical introduction of very great interest and importance. Prof. Lubosch is of opinion that comparative anatomy has two chief phases—pre-Darwinian and post-Darwinian. He is right, we think, when he seeks to explain why the great revolution in outlook, caused by the acceptance of the idea of evolution, must be attributed to Darwin and not to Lamarck. Darwin succeeded in impressing comparative anatomists by the

weight of evidence he thrust on them; Lamarck failed to influence his contemporaries because he presented his followers with ideas rather than with facts.

The author of the historical introduction documents his article with a long list of authorities. We are glad to note that Owen, Huxley and Ray Lankester are given honourable places in the history of comparative anatomy, but in the lists of authors—appended to this and to other articles—one is struck by the paucity of English names—even if we include representatives of the modern and thriving schools of America We know that in the development of comparative anatomy Germany has taken a large share, but we had no idea the share was so large as that which is reflected in the valuable lists of literature.

After the historical introduction follow two articles—one by Dr Adolf Naef of Cairo on the philosophy which underlies, or should underlie, all morphological enquiries and the other by Prof. Karl Peter of Greifswald on the significance of form and of organisation. More to our liking is the article by Prof. F. Alverdes of Marburg on the manner in which changes in structure and function are produced and how such changes are handed on from parents to offspring. The concluding chapter of the general part of vol. I is written by Prof. Viktor Fraz of Jena. It deals with the classification of the Vertebrates and with the evolution of vertebrate classes and orders. We note that this author brigades under the name *Chordata* three divisions—namely, Acrania, Tunicata and Vertebrata. The separation between Invertebrates and Vertebrates, which formerly seemed so sharp and wide, is becoming blurred and narrow. We note, too, that Gibbons are removed from their association with the Anthropomorphae and placed in a separate family—a just separation. Man's position in the order of Primates is left undetermined. One must conclude from some of the ideas expounded in this article, that the classification of the Vertebrata has become a subject which is too vast to receive adequate treatment from the pen of a single authority.

The part of vol. I which treats of the integumentary system is divided as follows: (1) A minute and well-illustrated account of the structure of skin in fishes and amphibiae by Prof. Rabl of Graz; (2) the structure of the skin in reptiles and birds by Dr B. Lange of Breslau; (3) an excellent account of the anatomy of the skin of mammals by Prof. S. Schumacher of Innsbruck; (4) a description of pigment-carrying cells in fishes, amphibians and reptiles by Prof. E. Ballowitz of Münster, Westphalia. Then follow a series of shorter but well-informed articles devoted to structures developed from the skin. Prof. J. E. V. Boas of Copenhagen describes the structure of hoofs, claws and nails; the horny and bony scales of Reptiles, Birds and Mammals; the structure of palmar and plantar pads; the structure and evolution of feathers. The scale formation in Fishes, Amphibia and Reptiles is described by Prof. B. Peyer of Zurich. The section on hair has been entrusted to the authoritative pen of Prof. J. C. H. Meijere of Amsterdam, who laments the diversity of opinion which still prevails as to factors which determine the stream line arrangement of hairy coverings but has to rest content by leaving matters as they are. Strangely enough, in enumerating the theories which have been adduced to explain the comparative hairlessness of Man's skin he does not allude to the interpretation given by his late colleague—perhaps the most satisfactory of all—namely that Man's comparative hairlessness represents an arrested development—the retention of a foetal state. The section devoted to the anatomy of skin glands has been entrusted to Prof. H. v. Eggeling of Breslau, while light-producing organs receive full treatment from Prof. A. Panners of Würzburg. A notable feature of this work is its list of references to literature. In the majority of instances the illustrations are well chosen and well executed. The second volume, which will appear soon, is devoted to an exposition of the anatomy of sense organs and of the peripheral nervous systems—somatic and visceral.

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